(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
31 December 2003 (31.12.2003)

PCT

(10) International Publication Number
WO 2004/001010 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number:
PCT/US2003/019760

(22) International Filing Date: 23 June 2003 (23.06.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/390,747	21 June 2002 (21.06.2002)	US
60/398,180	24 July 2002 (24.07.2002)	US
60/398,287	24 July 2002 (24.07.2002)	US

(71) Applicant *(for all designated States except US)*: **PTC THERAPEUTICS, INC.** [US/US]; 100 Corporate Court, Middlesex Business Center, South Plainfield, NJ 07080 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **WELCH, Ellen** [US/US]; 33 Hollow Brook Road, Califon, NJ 07830 (US). **ZHUO, Jin** [CN/US]; 22 Bolce Lane, Belle Meade, NJ 08502 (US).

(74) Agents: **CORUZZI, Laura, A.** et al.; Pennie & Edmonds LLP, 1155 Avenue of the Americas, New York, NY 10036 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

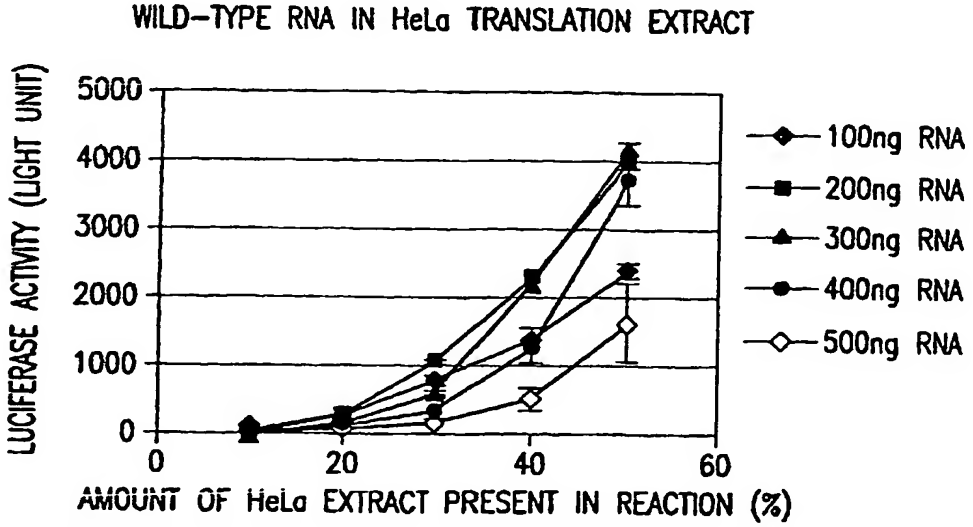
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

[Continued on next page]

(54) Title: METHODS FOR IDENTIFYING SMALL MOLECULES THAT MODULATE PREMATURE TRANSLATION TERMINATION AND NONSENSE MEDIATED mRNA DECAY



(57) Abstract: The present invention relates to methods for identifying compounds that modulate premature translation termination and/or nonsense-mediated mRNA decay by screening and identifying compounds that modulate the post-transcriptional expression of any gene with a premature translation stop codon. The invention particularly relates to using any gene encoding a premature stop codon to identify compounds that modulate premature translation termination and/or nonsense-mediated mRNA decay. A compound that modulates premature translation termination and/or nonsense-mediated mRNA decay of a target gene is identified using standard methods known in the art to measure changes in translation or mRNA stability of the gene product or mRNA of the gene with the premature stop codon. The methods of the present invention provide a simple, sensitive assay for high-throughput screening of libraries of compounds to identify pharmaceutical leads.

WO 2004/001010 A2

METHODS FOR IDENTIFYING SMALL MOLECULES THAT MODULATE PREMATURE TRANSLATION TERMINATION AND NONSENSE MEDIATED mRNA DECAY

This application is entitled to and claims priority benefit to U.S. Provisional Patent Application No. 60/390,747, filed June 21, 2002, U.S. Provisional Patent Application No. 60/398,180, filed July 24, 2002 and U.S. Provisional Patent Application No. 60/398,287, filed July 24, 2002, each of which are incorporated herein by reference in their entirety.

1. INTRODUCTION

The present invention relates to a method for screening and identifying compounds that modulate premature translation termination and/or nonsense-mediated messenger ribonucleic acid ("mRNA") decay by screening and identifying compounds that modulate the post-transcriptional expression of any gene with a premature translation stop codon. A compound that modulates premature translation termination and/or nonsense-mediated mRNA decay of a target gene is identified using standard methods known in the art to measure changes in translation or mRNA stability of the gene product or mRNA of the gene with the premature stop codon. The methods of the present invention provide a simple, sensitive assay for high-throughput screening of libraries of compounds to identify pharmaceutical leads.

2. BACKGROUND OF THE INVENTION

Protein synthesis encompasses the processes of translation initiation, elongation, and termination, each of which has evolved to occur with great accuracy and has the capacity to be a regulated step in the pathway of gene expression. Recent studies, including those suggesting that events at termination may regulate the ability of ribosomes to recycle to the start site of the same mRNA, have underscored the potential of termination to regulate other aspects of translation. The RNA triplets UAA, UAG, and UGA are non-coding and promote translational termination. Termination starts when one of the three termination codons enters the A site of the ribosome signaling the polypeptide chain release factors to bind and recognize the termination signal. Subsequently, the ester bond between

the 3' nucleotide of the transfer RNA ("tRNA") located in the ribosome's P site and the nascent polypeptide chain is hydrolyzed, the completed polypeptide chain is released, and the ribosome subunits are recycled for another round of translation.

Nonsense-mediated mRNA decay is a surveillance mechanism that minimizes the translation and regulates the RNA stability of nonsense RNAs that contain chain termination mutations (see, *e.g.*, Hentze & Kulozik, 1999, Cell 96:307-310; Culbertson, 1999, Trends in Genetics 15:74-80; Li & Wilkinson, 1998, Immunity 8:135-141; and Ruiz-Echevarria *et al.*, 1996, Trends in Biological Sciences, 21:433-438). Chain termination mutations are caused by a base substitution or frameshift mutation changes a codon into a termination codon, *i.e.*, a stop codon that causes translational termination. In nonsense-mediated mRNA decay, mRNAs with premature stop codons are subject to degradation. In some cases, a truncated protein is produced if the premature stop codon is located near the end of an open reading frame.

Certain classes of known antibiotics have been characterized and found to interact with RNA. For example, the antibiotic thiostreptone binds tightly to a 60-mer from ribosomal RNA (Cundliffe *et al.*, 1990, in The Ribosome: Structure, Function & Evolution (Schlessinger *et al.*, eds.) American Society for Microbiology, Washington, D.C. pp. 479-490). Bacterial resistance to various antibiotics often involves methylation at specific rRNA sites (Cundliffe, 1989, Ann. Rev. Microbiol. 43:207-233). Aminoglycosidic aminocyclitol (aminoglycoside) antibiotics and peptide antibiotics are known to inhibit group I intron splicing by binding to specific regions of the RNA (von Ahsen *et al.*, 1991, Nature (London) 353:368-370). Some of these same aminoglycosides have also been found to inhibit hammerhead ribozyme function (Stage *et al.*, 1995, RNA 1:95-101). In addition, certain aminoglycosides and other protein synthesis inhibitors have been found to interact with specific bases in 16S rRNA (Woodcock *et al.*, 1991, EMBO J. 10:3099-3103). An oligonucleotide analog of the 16S rRNA has also been shown to interact with certain aminoglycosides (Purohit *et al.*, 1994, Nature 370:659-662). A molecular basis for hypersensitivity to aminoglycosides has been found to be located in a single base change in mitochondrial rRNA (Hutchin *et al.*, 1993, Nucleic Acids Res. 21:4174-4179). Aminoglycosides have also been shown to inhibit the interaction between specific structural RNA motifs and the corresponding RNA binding protein. Zapp *et al.* (Cell, 1993, 74:969-978) has demonstrated that the aminoglycosides neomycin B, lividomycin A, and tobramycin can block the binding of Rev, a viral regulatory protein required for viral gene expression, to its viral recognition element in the IIB (or RRE) region of HIV RNA. This

blockage appears to be the result of competitive binding of the antibiotics directly to the RRE RNA structural motif.

Aminoglycosides have also been found to promote nonsense suppression (see, *e.g.*, Bedwell *et al.*, 1997, Nat. Med. 3:1280-1284 and Howard *et al.*, 1996, Nat. Med. 2:467-469). Nonsense mutations cause approximately 10 to 30 percent of the individual cases of virtually all inherited diseases. Although nonsense mutations inhibit the synthesis of a full length protein to one percent or less of wild-type levels, minimally boosting the expression levels of the full length protein to between five and fifteen percent of normal levels can greatly reduce the severity or eliminate the disease. Clinical approaches that target the translation termination event to promote nonsense suppression have recently been described for model systems of cystic fibrosis and muscular dystrophy. Gentamicin is an aminoglycoside antibiotic that causes translational misreading and allowed the insertion of amino acids at the site of the nonsense codon in models of cystic fibrosis, Hurlers Syndrome, and muscular dystrophy (see, *e.g.*, Barton-Davis *et al.*, 1999, J. Clin. Invest. 104:375-381). These results strongly suggest that drugs that promote nonsense suppression by altering translation termination efficiency of a premature termination codon can be therapeutically valuable in the treatment of diseases caused by nonsense mutations.

Citation or identification of any reference in Section 2 of this application is not an admission that such reference is available as prior art to the present invention.

3. SUMMARY OF THE INVENTION

The present invention provides methods for identifying a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay. In particular, the invention provides methods for identifying a compound that suppresses premature translation termination and/or nonsense-mediated mRNA decay. The invention encompasses the use of the compounds identified utilizing the methods of the invention for the prevention, treatment, management or amelioration of a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay, or a symptom thereof.

The invention provides cell-based and cell-free assays for the identification of a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay. In general, the level of expression of a reporter gene product past the premature termination codon and/or activity of such a gene product in the reporter gene based-assays described herein is indicative of the effect of the compound on premature

translation termination and/or nonsense-mediated mRNA decay. The reporter gene-based assays described herein for the identification of compounds that modulate premature translation termination and/or nonsense-mediated mRNA decay are well suited for high-throughput screening.

The reporter gene cell-based assays may be conducted by contacting a compound with a cell containing a nucleic acid sequence comprising a reporter gene, wherein the reporter gene comprises a premature stop codon or nonsense mutation, and measuring the expression of the reporter gene. The reporter gene cell-based assays may also be conducted by: (a) contacting a compound with a cell containing a first nucleic acid sequence and a second nucleic acid sequence, wherein the first nucleic acid sequence comprises a regulatory element operably linked to a reporter gene and the second nucleic acid sequence comprises a nucleotide sequence with a premature stop codon or nonsense mutation that encodes a regulatory protein that binds to the regulatory element of the first nucleic acid sequence and regulates the expression of the reporter gene; and (b) measuring the expression of the reporter gene.

The reporter gene cell-based assays may also be conducted by: (a) contacting a compound with a cell containing a first nucleic acid sequence, a second nucleic acid sequence and a third nucleic acid sequence, wherein (i) the first nucleic acid sequence comprises a nucleotide sequence encoding a first fusion protein comprising a DNA binding domain and a first protein, the nucleotide sequence of the DNA binding domain or the first protein containing a premature stop codon or nonsense mutation, (ii) the second nucleic acid sequence comprises a nucleotide sequence encoding a second fusion protein comprising an activation domain and a second protein, the second protein interacting with the first protein to produce a regulatory protein, and (iii) the third nucleic acid sequence comprises a regulatory element operably linked to a reporter gene, the expression of the reporter gene being regulated by the binding of the regulatory protein to the regulatory element: and (b) measuring the expression of the reporter gene. Further, the reporter gene cell-based assays may be conducted by: (a) contacting a compound with a cell containing a first nucleic acid sequence, a second nucleic acid sequence and a third nucleic acid sequence, wherein (i) the first nucleic acid sequence comprises a nucleotide sequence encoding a first fusion protein comprising a DNA binding domain and a first protein, (ii) the second nucleic acid sequence comprises a nucleotide sequence encoding a second fusion protein comprising an activation domain and a second protein, the nucleotide sequence of the activation domain or the second protein containing a premature stop codon or nonsense mutation, and the second

protein interacting with the first protein to produce a regulatory protein, and (iii) the third nucleic acid sequence comprises a regulatory element operably linked to a reporter gene, the expression of the reporter gene being regulated by the binding of the regulatory protein to the regulatory element: and (b) measuring the expression of the reporter gene.

The reporter gene cell-free assays may be conducted by contacting a compound with a cell-free extract and a nucleic acid sequence comprising a reporter gene, wherein the reporter gene comprises a premature stop codon or nonsense mutation, and measuring the expression of the reporter gene. The reporter gene cell-free assays may also be conducted by contacting a compound with a cell-free extract and an *in vitro* transcribed RNA of a reproter gene, wherein the RNA product contains a premature stop codon or nonsense mutation, and measuring the expression of the protein encoded by the RNA product. The reporter gene cell-free assays may also be conducted by: (a) contacting a compound with a cell-free extract, a first nucleic acid sequence and a second nucleic acid sequence, wherein the first nucleic acid sequence comprises a regulatory element operably linked to a reporter gene and the second nucleic acid sequence comprises a nucleotide sequence with a premature stop codon or nonsense mutation that encodes a regulatory protein that binds to the regulatory element of the first nucleic acid sequence and regulates the expression of the reporter gene; and (b) measuring the expression of the reporter gene.

The reporter gene cell-free assays may also be conducted by: (a) contacting a compound with a cell-free extract, a first nucleic acid sequence, a second nucleic acid sequence and a third nucleic acid sequence, wherein (i) the first nucleic acid sequence comprises a nucleotide sequence encoding a first fusion protein comprising a DNA binding domain and a first protein, the nucleotide sequence of the DNA binding domain or the first protein containing a premature stop codon or nonsense mutation, (ii) the second nucleic acid sequence comprises a nucleotide sequence encoding a second fusion protein comprising an activation domain and a second protein, the second protein interacting with the first protein to produce a regulatory protein, and (iii) the third nucleic acid sequence comprises a regulatory element operably linked to a reporter gene, the expression of the reporter gene being regulated by the binding of the regulatory protein to the regulatory element: and (b) measuring the expression of the reporter gene. The reporter gene cell-free assays may also be conducted by: (a) contacting a compound with a cell-free extract, a first nucleic acid sequence, a second nucleic acid sequence and a third nucleic acid sequence, wherein (i) the first nucleic acid sequence comprises a nucleotide sequence encoding a first fusion protein comprising a DNA binding domain and a first protein, (ii) the second nucleic acid sequence

comprises a nucleotide sequence encoding a second fusion protein comprising an activation domain and a second protein, the nucleotide sequence of the activation domain or the second protein containing a premature stop codon or nonsense mutation, and the second protein interacting with the first protein to produce a regulatory protein, and (iii) the third nucleic acid sequence comprises a regulatory element operably linked to a reporter gene, the expression of the reporter gene being regulated by the binding of the regulatory protein to the regulatory element; and (b) measuring the expression of the reporter gene.

In the cell-based and cell-free reporter gene assays described herein, the alteration in reporter gene expression or activity relative to a previously determined reference range, or to the expression or activity of the reporter gene in the absence of the compound or the presence of an appropriate control (*e.g.*, a negative control) indicates that a particular compound modulates premature translation termination and/or nonsense-mediated mRNA decay. In particular, a decrease in reporter gene expression or activity relative to a previously determined reference range, or to the expression in the absence of the compound or the presence of an appropriate control (*e.g.*, a negative control) may, depending upon the parameters of the reporter gene assay, indicate that a particular compound reduces or suppresses premature translation termination and/or nonsense-mediated mRNA decay. In contrast, an increase in reporter gene expression or activity relative to a previously determined reference range, or to the expression in the absence of the compound or the presence of an appropriate control (*e.g.*, a negative control) may, depending upon the parameters of the reporter gene-based assay, indicate that a particular compound enhances premature translation termination and/or nonsense-mediated mRNA decay.

The invention relates to the identification of compounds that modulate premature translation termination or nonsense-mediated mRNA decay, using, in some instances, a reporter based assay. The invention provides for the identification of compounds that modulated premature translation termination via a nonsense stop codon in a nucleic acids. Such nucleic acids include, but are not limited to, DNA and RNA. In a more certain embodiment, the nucleic acid is RNA. In another embodiment, the nucleic acid is single stranded. In other embodiments, the nucleic acids are single stranded. In yet other embodiments, the nucleic acids are more than single stranded, e.g., double, triple or quadruple stranded.

In one embodiment, the invention provides a method for identifying a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay, said method comprising: (a) expressing a nucleic acid sequence comprising a

reporter gene in a cell, wherein the reporter gene comprises a premature stop codon; (b) contacting said cell with a member of a library of compounds; and (c) detecting the expression of said reporter gene, wherein a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay is identified if the expression of said reporter gene in the presence of a compound is altered relative to a previously determined reference range, or the expression of said reporter gene in the absence of the compound or the presence of an appropriate control (*e.g.*,. a negative control such as phosphate buffered saline).

In another embodiment, the invention provides a method for identifying a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay, said method comprising: (a) contacting a member of a library of compounds with a cell containing a nucleic acid sequence comprising a reporter gene, wherein the reporter gene comprises a premature stop codon; and (b) detecting the expression of said reporter gene, wherein a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay is identified if the expression of said reporter gene in the presence of a compound is altered relative to a previously determined reference range, or the expression of said reporter gene in the absence of said compound or the presence of an appropriate control (*e.g.*, a negative control).

In another embodiment, the invention provides a method for identifying a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay, said method comprising: (a) contacting a member of a library of compounds with a cell-free extract and a nucleic acid sequence comprising a reporter gene, wherein the reporter gene comprises a premature stop codon; and (b) detecting the expression of said reporter gene, wherein a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay is identified if the expression of said reporter gene in the presence of a compound is altered relative to a previously determined reference range, or the expression of said reporter gene in the absence of said compound or the presence of an appropriate control (*e.g.*, a negative control). In accordance with this embodiment, the cell-extract is preferably isolated from cells that have been incubated at about 0°C to about 10°C and/or an S10 to S30 cell-free extract.

In another embodiment, the invention provides a method for identifying a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay, said method comprising: (a) contacting a member of a library of compounds with a cell containing a first nucleic acid sequence and a second nucleic acid sequence,

wherein the first nucleic acid sequence comprises a regulatory element operably linked to a reporter gene and the second nucleic acid sequence comprises a nucleotide sequence with a premature stop codon that encodes a regulatory protein that binds to the regulatory element of the first nucleic acid sequence and regulates the expression of the reporter gene; and (b) detecting the expression of the reporter gene, wherein a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay is identified if the expression of said reporter gene in the presence of a compound is altered relative to a previously determined reference range, or the expression of said reporter gene in the absence of said compound or the presence of an appropriate control (*e.g.*, a negative control).

In another embodiment, the invention provides a method for identifying a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay, said method comprising: (a) contacting a member of a library of compounds with a cell containing a first nucleic acid sequence, a second nucleic acid sequence and a third nucleic acid sequence, wherein (i) the first nucleic acid sequence comprises a nucleotide sequence encoding a first fusion protein comprising a DNA binding domain and a first protein, the nucleotide sequence of the DNA binding domain or the first protein comprising a premature stop codon, (ii) the second nucleic acid sequence comprises a nucleotide sequence encoding a second fusion protein comprising an activation domain and a second protein, the second protein interacting with the first protein to produce a regulatory protein, and (iii) the third nucleic acid sequence comprises a regulatory element operably linked to a reporter gene, the expression of the reporter gene being regulated by the binding of the regulatory protein to the regulatory element; and (b) detecting the expression of the reporter gene, wherein a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay is identified if the expression of said reporter gene in the presence of a compound is altered relative to a previously determined reference range, or the expression of said reporter gene in the absence of said compound or the presence of an appropriate control (*e.g.*, a negative control).

In another embodiment, the invention provides a method for identifying a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay, said method comprising: (a) contacting a member of a library of compounds with a cell containing a first nucleic acid sequence, a second nucleic acid sequence and a third nucleic acid sequence, wherein (i) the first nucleic acid sequence comprises a nucleotide sequence encoding a first fusion protein comprising a DNA binding domain and

a first protein, (ii) the second nucleic acid sequence comprises a nucleotide sequence encoding a second fusion protein comprising an activation domain and a second protein, the nucleotide sequence of the activation domain or the second protein containing a premature stop codon, and the second protein interacting with the first protein to produce a regulatory protein, and (iii) the third nucleic acid sequence comprises a regulatory element operably linked to a reporter gene, the expression of the reporter gene being regulated by the binding of the regulatory protein to the regulatory element; and (b) detecting the expression of the reporter gene, wherein a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay is identified if the expression of said reporter gene in the presence of a compound is altered relative to a previously determined reference range, or the expression of said reporter gene in the absence of said compound or the presence of an appropriate control (*e.g.*, a negative control).

In another embodiment, the invention provides a method for identifying a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay, said method comprising: (a) contacting a member of a library of compounds with a cell-free extract, a first nucleic acid sequence and a second nucleic acid sequence, wherein the first nucleic acid sequence comprises a regulatory element operably linked to a reporter gene and the second nucleic acid sequence comprises a nucleotide sequence with a premature stop codon that encodes a regulatory protein that binds to the regulatory element of the first nucleic acid sequence and regulates the expression of the reporter gene; and (b) detecting the expression of the reporter gene, wherein a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay is identified if the expression of said reporter gene in the presence of a compound is altered relative to a previously determined reference range, or the expression of said reporter gene in the absence of said compound or the presence of an appropriate control (*e.g.*, a negative control).

In another embodiment, the invention provides a method for identifying a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay, said method comprising: (a) contacting a member of a library of compounds with a cell-free extract, a first nucleic acid sequence, a second nucleic acid sequence and a third nucleic acid sequence, wherein (i) the first nucleic acid sequence comprises a nucleotide sequence encoding a first fusion protein comprising a DNA binding domain and a first protein, the nucleotide sequence of the DNA binding domain or the first protein comprising a premature stop codon, (ii) the second nucleic acid sequence comprises a

nucleotide sequence encoding a second fusion protein comprising an activation domain and a second protein, the second protein interacting with the first protein to produce a regulatory protein, and (iii) the third nucleic acid sequence comprises a regulatory element operably linked to a reporter gene, the expression of the reporter gene being regulated by the binding of the regulatory protein to the regulatory element; and (b) detecting the expression of the reporter gene, wherein a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay is identified if the expression of said reporter gene in the presence of a compound is altered relative to a previously determined reference range, or the expression of said reporter gene in the absence of said compound or the presence of an appropriate control (*e.g.*, a negative control).

In another embodiment, the invention provides a method for identifying a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay, said method comprising: (a) contacting a member of a library of compounds with a cell-free extract, a first nucleic acid sequence, a second nucleic acid sequence and a third nucleic acid sequence, wherein (i) the first nucleic acid sequence comprises a nucleotide sequence encoding a first fusion protein comprising a DNA binding domain and a first protein, (ii) the second nucleic acid sequence comprises a nucleotide sequence encoding a second fusion protein comprising an activation domain and a second protein, the nucleotide sequence of the activation domain or the second protein containing a premature stop codon, and the second protein interacting with the first protein to produce a regulatory protein, and (iii) the third nucleic acid sequence comprises a regulatory element operably linked to a reporter gene, the expression of the reporter gene being regulated by the binding of the regulatory protein to the regulatory element; and (b) detecting the expression of the reporter gene, wherein a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay is identified if the expression of said reporter gene in the presence of a compound is altered relative to a previously determined reference range, or the expression of said reporter gene in the absence of said compound or the presence of an appropriate control (*e.g.*, a negative control).

In accordance with the invention, the step of contacting a compound with a cell, or cell-free extract and a nucleic acid sequence in the reporter gene-based assays described herein is preferably conducted in an aqueous solution comprising a buffer and a combination of salts (such as KCl, NaCl and/or $MgCl_2$). The optimal concentration of each salt used in the aqueous solution is dependent on, *e.g.*, the protein, polypeptide or peptide encoded by the nucleic acid sequence (*e.g.*, the regulatory protein) and the compounds used,

and can be determined using routine experimentation. In a specific embodiment, the aqueous solution approximates or mimics physiologic conditions. In another specific embodiment, the aqueous solution further comprises a detergent or a surfactant.

The assays of the present invention can be performed using different incubation times. In the a cell-based system, the cell and a compound or a member of a library of compounds may be incubated together for at least 0.2 hours, 0.25 hours, 0.5 hours, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 8 hours, 10 hours, 12 hours, 18 hours, at least 1 day, at least 2 days or at least 3 days before the expression and/or activity of a reporter gene is measured. In a cell-free system, the cell-free extract and the nucleic acid sequence(s) (*e.g.*, a reporter gene) can be incubated together before the addition of a compound or a member of a library of compounds. In certain embodiments, the cell-free extract are incubated with a nucleic acid sequence(s) (*e.g.*, a reporter gene) before the addition of a compound or a member of a library of compounds for at least 0.2 hours, 0.25 hours, 0.5 hours, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 8 hours, 10 hours, 12 hours, 18 hours, or at least 1 day. In other embodiments, the cell-free extract, or the nucleic acid sequence(s) (*e.g.*, a reporter gene) is incubated with a compound or a member of a library of compounds before the addition of the nucleic acid sequence(s) (*e.g.*, a reporter gene), or the cell-free extract, respectively. In certain embodiments, a compound or a member of a library of compounds is incubated with a nucleic acid sequence(s) (*e.g.*, a reporter gene) or cell-free extract before the addition of the remaining component, *i.e.*, cell-free extract, or a nucleic acid sequence(s) (*e.g.*, a reporter gene), respectively, for at least 0.2 hours, 0.25 hours, 0.5 hours, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 8 hours, 10 hours, 12 hours, 18 hours, or at least 1 day. Once the reaction vessel comprises the components, *i.e.*, a compound or a member of a library of compounds, the cell-free extract and the nucleic acid sequence(s) (*e.g.*, a reporter gene), the reaction may be further incubated for at least 0.2 hours, 0.25 hours, 0.5 hours, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 8 hours, 10 hours, 12 hours, 18 hours, or at least 1 day.

The progress of the reaction in the reporter gene-based assays can be measured continuously. Alternatively, time-points may be taken at different times of the reaction to monitor the progress of the reaction in the reporter gene-based assays.

The reporter gene-based assays described herein may be conducted in a cell genetically engineered to express a reporter gene or *in vitro* utilizing a cell-free extract. Any cell or cell line of any species well-known to one of skill in the art may be utilized in accordance with the methods of the invention. Further, a cell-free extract may be derived

from any cell or cell line of any species well-known to one of skill in the art. Examples of cells and cell types include, but are not limited to, human cells, cultured mouse cells, cultured rat cells or Chinese hamster ovary ("CHO") cells.

The reporter gene constructs utilized in the reporter gene-based assays described herein may comprise the coding region of a reporter gene and a premature stop codon that results in premature translation termination and/or nonsense-mediated mRNA decay. Preferably, the premature stop codon is N-terminal to the native stop codon of the reporter gene and is located such that the suppression of the premature stop codon is readily detectable. In a specific embodiment, a reporter gene construct utilized in the reporter gene-based assays described herein comprises the coding region of a reporter gene containing a premature stop codon at least 15 nucleotides, preferably 25 to 50 nucleotides, 50 to 75 nucleotides or 75 to 100 nucleotides from the start codon in the open reading frame of the reporter gene. In another embodiment, a reporter gene construct utilized in the reporter gene-based assays described herein comprises the coding region of a reporter gene containing a premature stop codon at least 15 nucleotides, preferably 25 to 50 nucleotides, 50 to 75 nucleotides, 75 to 100 nucleotides, or 100 to 150 nucleotides from the native stop codon in the open reading frame of the reporter gene. In another embodiment, a reporter gene construct utilized in the reporter gene-based assays described herein comprises the coding region of a reporter gene containing a UAG and/or UGA premature stop codon. In yet another embodiment, a reporter gene construct utilized in the reporter gene based assays described herein comprises the coding region of a reporter gene, containing a premature stop codon in the context of UGAA, UGAC, UGAG, UGAU, UAGA, UAGC, UAGG, UAGU, UAAA, UAAC, UAAG or UAAU..

Alternatively, the reporter gene constructs utilized in the reporter gene-based assays described herein comprise a regulatory element that is responsive to a regulatory protein encoded by a nucleic acid sequence containing a premature stop codon. Preferably, the premature stop codon in the nucleotide sequence of a regulatory protein or a component or subunit thereof is N-terminal to the native stop codon of the regulatory protein or component or subunit thereof and the location of the premature stop codon is such that it alters the biological activity of the regulatory protein (*e.g.*, the ability of the regulatory protein to bind to its regulatory element). In a specific embodiment, the premature stop codon in the nucleotide sequence of a regulatory protein or a component or subunit thereof is at least 15 nucleotides preferably 25 to 50 nucleotides, 50 to 75 nucleotides or 75 to 100 nucleotides from the start codon in the open reading frame of the regulatory protein,

component or subunit thereof. In another embodiment, the premature stop codon in the nucleotide sequence of a regulatory protein or a component or subunit thereof is at least 15 nucleotides, preferably 25 to 50 nucleotides, 50 to 75 nucleotides, 75 to 100 nucleotides, or 100 to 150 nucleotides from the native stop codon in the open reading frame of the regulatory protein, component or subunit thereof. In another embodiment, the premature stop codon in the nucleotide sequence of regulatory protein or a component or subunit thereof is UAG or UGA. Any reporter gene well-known to one of skill in the art may be utilized in the reporter gene constructs described herein. Examples of reporter genes include, but are not limited to, the gene encoding firefly luciferase, the gene coding renilla luciferase, the gene encoding click beetle luciferase, the gene encoding green fluorescent protein, the gene encoding yellow fluorescent protein, the gene encoding red fluorescent protein, the gene encoding cyan fluorescent protein, the gene encoding blue fluorescent protein, the gene encoding beta-galactosidase, the gene encoding beta-glucoronidase, the gene encoding beta-lactamase, the gene encoding chloramphenicol acetyltransferase, and the gene encoding alkaline phosphatase.

The compounds utilized in the assays described herein may be members of a library of compounds. In specific embodiment, the compound is selected from a combinatorial library of compounds comprising peptoids; random biooligomers; diversomers such as hydantoins, benzodiazepines and dipeptides; vinylogous polypeptides; nonpeptidal peptidomimetics; oligocarbamates; peptidyl phosphonates; peptide nucleic acid libraries; antibody libraries; carbohydrate libraries; and small organic molecule libraries. In a preferred embodiment, the small organic molecule libraries are libraries of benzodiazepines, isoprenoids, thiazolidinones, metathiazanones, pyrrolidines, morpholino compounds, or diazepindiones.

In certain embodiments, the compounds are screened in pools. Once a positive pool has been identified, the individual compounds of that pool are tested separately. In certain embodiments, the pool size is at least 2, at least 5, at least 10, at least 25, at least 50, at least 75, at least 100, at least 150, at least 200, at least 250, or at least 500 compounds.

Once a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay is identified, the structure of the compound may be determined utilizing well-known techniques or by referring to a predetermined code. For example, the structure of the compound may be determined by mass spectroscopy, NMR, vibrational spectroscopy, or X-ray crystallography.

A compound identified in accordance with the methods of the invention may directly bind to the mRNA translation machinery. Alternatively, a compound identified in accordance with the methods of invention may bind to the premature stop codon. A compound identified in accordance with the methods of invention may also disrupt an interaction between a premature stop codon and the mRNA translation machinery. In a preferred embodiment, a compound identified in accordance with the methods of the invention suppresses premature translation termination and/or nonsense-mediated mRNA decay of a gene encoding a protein, polypeptide or peptide whose expression is beneficial to a subject. In another preferred embodiment, a compound identified in accordance with the methods of the invention increases premature translation termination and/or nonsense-mediated mRNA decay of a gene encoding a protein, polypeptide or peptide whose expression is detrimental to a subject. In a specific embodiment, a compound identified in accordance with the methods of the invention preferentially or differentially modulates premature translation termination and/or nonsense-mediated mRNA decay of a specific nucleotide sequence of interest relative to another nucleotide sequence, as measured by an assay described herein or well known to one of skill in the art under the same or similar assay conditions.

In a specific embodiment, a compound identified in accordance with the invention suppresses premature translation termination or nonsense-mediated mRNA decay of a specific nucleotide sequence of interest by at least 5%, preferably at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%, relative to an appropriate control (*e.g.*, a negative control such as PBS), in an assay described herein under the same or similar assay conditions. In accordance with this embodiment, preferably, the compound differentially or preferentially suppresses the nucleotide sequence of interest relative to another nucleotide sequence.

In certain embodiments of the invention, the compound identified using the assays described herein is a small molecule. In a preferred embodiment, the compound identified using the assays described herein is not known to affect premature translation termination and/or nonsense-mediated mRNA decay of a nucleic acid sequence, in particular a nucleic acid sequence of interest. In another preferred embodiment, the compound identified using the assays described herein has not been used as or suggested to be used in the prevention, treatment, management and/or amelioration of a disorder

associated with, characterized by or caused by a premature stop codon. In another preferred embodiment, the compound identified using the assays described herein has not been used as or suggested to be used in the prevention, treatment, management and/or amelioration of a particular disorder described herein.

A compound identified in accordance with the methods of the invention may be tested in *in vitro* and/or *in vivo* assays well-known to one of skill in the art or described herein to determine the prophylactic or therapeutic effect of a particular compound for a particular disorder. In particular, a compound identified utilizing the assays described herein may be tested in an animal model to determine the efficacy of the compound in the prevention, treatment or amelioration of a disorder associated with, characterized by or caused by a premature stop codon, or a disorder described herein, or a symptom thereof. In addition, a compound identified utilizing the assays described herein may be tested for its toxicity in *in vitro* and/or *in vivo* assays well-known to one of skill in the art.

The invention provides for methods for preventing, treating, managing or ameliorating a disorder associated with, characterized by or caused by a premature stop codon or a symptom thereof, said method comprising administering to a subject in need thereof a therapeutically or prophylactically effective amount of a compound, or a pharmaceutically acceptable salt thereof, identified according to the methods described herein.

The present invention may be understood more fully by reference to the detailed description and examples, which are intended to illustrate non-limiting embodiments of the invention.

3.1. Terminology

As used herein, the term "compound" refers to any agent or complex that is being tested for its ability to modulate premature translation termination and/or nonsense-mediated mRNA decay or has been identified as modulating premature translation termination and/or nonsense-mediated mRNA decay.

As used herein, the terms "disorder" and "disease" are to refer to a condition in a subject. In a specific embodiment, the terms disease and disorder refer to a condition in a subject that is associated with, characterized by, or caused by premature translation termination and/or nonsense-mediated mRNA decay of one or more gene products. Non-limiting examples of such disease and disorders are described herein below.

As used herein, the term "effective amount" refers to the amount of a compound which is sufficient to (i) reduce or ameliorate the progression, severity and/or

duration of a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay, or one or more symptoms thereof, (ii) prevent the development, recurrence or onset of a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay, or one or more symptoms thereof, (iii) prevent the advancement of a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay, or one or more symptoms thereof, or (iv) enhance or improve the therapeutic(s) effect(s) of another therapy.

As used herein, the term "host cell" includes a particular subject cell transfected with a nucleic acid molecule and the progeny or potential progeny of such a cell. Progeny of such a cell may not be identical to the parent cell transfected with the nucleic acid molecule due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome.

As used herein, the term "in combination" refers to the use of more than one therapy (*e.g.*, prophylactic and/or therapeutic agents). The use of the term "in combination" does not restrict the order in which therapies (*e.g.*, prophylactic and/or therapeutic agents) are administered to a subject with a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay. A first therapy (*e.g.*, a prophylactic or therapeutic agent such as a compound identified in accordance with the methods of the invention) can be administered prior to (*e.g.*, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (*e.g.*, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy (*e.g.*, a prophylactic or therapeutic agent such as a chemotherapeutic agent or a TNF-α antagonist) to a subject with a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay.

As used herein, the term "library" in the context of compounds refers to a plurality of compounds. A library can be a combinatorial library, *e.g.*, a collection of compounds synthesized using combinatorial chemistry techniques, or a collection of unique chemicals of low molecular weight (less than 1000 daltons) that each occupy a unique three-dimensional space.

As used herein, the terms "manage", "managing" and "management" refer to the beneficial effects that a subject derives from a therapy (*e.g.*, a prophylactic or therapeutic agent) which does not result in a cure of the disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay. In certain embodiments, a subject is administered one or more therapies to "manage" a disease or disorder so as to prevent the progression or worsening of the disease or disorder.

As used herein, the phrase "modulation of premature translation termination and/or nonsense-mediated mRNA decay" refers to the regulation of gene expression by altering the level of nonsense suppression. For example, if it is desirable to increase production of a defective protein encoded by a gene with a premature stop codon, *i.e.*, to permit readthrough of the premature stop codon of the disease gene so translation of the gene can occur, then modulation of premature translation termination and/or nonsense-mediated mRNA decay entails up-regulation of nonsense suppression. Conversely, if it is desirable to promote the degradation of an mRNA with a premature stop codon, then modulation of premature translation termination and/or nonsense-mediated mRNA decays entails down-regulation of nonsense suppression.

As used herein, the terms "non-responsive" and refractory" describe patients treated with a currently available therapy (*e.g.*, prophylactic or therapeutic agent) for a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay (*e.g.*, cancer), which is not clinically adequate to relieve one or more symptoms associated with such disorder. Typically, such patients suffer from severe, persistently active disease and require additional therapy to ameliorate the symptoms associated with their disoder.

As used herein, "nonsense-mediated mRNA decay" refers to any mechanism that mediates the decay of mRNAs containing a premature translation termination codon.

As used herein, a "nonsense mutation" is a point mutation changing a codon corresponding to an amino acid to a stop codon.

As used herein, "nonsense suppression" refers to the inhibition or suppression of premature translation termination and/or nonsense-mediated mRNA decay.

The terms "nucleic acid," "nucleic acid sequence," "nucleotide sequence," and analagous terms as used herein include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), combinations of DNA and RNA molecules of hybrid DNA/RNA molecules, and analogs of DNA or RNA molecules. Such analogs can be

generated using, for example, nucleotide analogs, which include, but are not limited to, inosine or tritylated bases. Such analogs can also comprise DNA or RNA molecules comprising modified backbones that lend beneficial attributes to the molecules, such as, for example, nuclease resistance or an increased ability to cross cellular membranes. The nucleic acids, nucleic acid sequences or nucleotide sequences can be single-stranded, double-stranded, may contain both single-stranded and double-stranded portions, and may contain triple-stranded portions, but prefereably is double-stranded DNA. In one embodiment, the nucleotide sequences comprise a contiguous open reading frame encoding a reporter gene, *e.g.*, a cDNA molecule.

As used herein, the phrase "pharmaceutically acceptable salt(s)," includes, but is not limited to, salts of acidic or basic groups that may be present in compounds identified using the methods of the present invention. Compounds that are basic in nature are capable of forming a wide variety of salts with various inorganic and organic acids. The acids that can be used to prepare pharmaceutically acceptable acid addition salts of such basic compounds are those that form non-toxic acid addition salts, *i.e.*, salts containing pharmacologically acceptable anions, including but not limited to sulfuric, citric, maleic, acetic, oxalic, hydrochloride, hydrobromide, hydroiodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, isonicotinate, acetate, lactate, salicylate, citrate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate and pamoate (*i.e.*, 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. Compounds that include an amino moiety may form pharmaceutically acceptable salts with various amino acids, in addition to the acids mentioned above. Compounds that are acidic in nature are capable of forming base salts with various pharmacologically acceptable cations. Examples of such salts include alkali metal or alkaline earth metal salts and, particularly, calcium, magnesium, sodium lithium, zinc, potassium, and iron salts.

As used herein, "premature translation termination" refers to the result of a mutation that changes a codon corresponding to an amino acid to a stop codon.

As used herein, the terms "prevent", " preventing" and "prevention" refer to the prevention of the development, recurrence or onset of a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay or one or more symptoms thereof resulting from the administration of one or more compounds identified in accordance the methods of the invention or the

administration of a combination of such a compound and a known therapy for such a disorder.

As used herein, the term "previously determined reference range" refers to a reference range for the readout of a particular assay. In a specific embodiment, the term refers to a reference range for the expression of a reporter gene and/or the activity of a reporter gene product by a particular cell or in a particular cell-free extract. Each laboratory will establish its own reference range for each particular assay, each cell type and each cell-free extract. In a preferred embodiment, at least one positive control and at least one negative control are included in each batch of compounds analyzed.

As used herein, the terms "prophylactic agent" and "prophylactic agents" refer to any agent(s) which can be used in the prevention of a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay. In certain embodiments, the term "prophylactic agent" refers to a compound identified in the screening assays described herein. In certain other embodiments, the term "prophylactic agent" refers to an agent other than a compound identified in the screening assays described herein which is known to be useful for, or has been or is currently being used to prevent or impede the onset, development and/or progression of a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay or one or more symptoms thereof.

As used herein, the phrase "prophylactically effective amount" refers to the amount of a therapy (*e.g.*, a prophylactic agent) which is sufficient to result in the prevention of the development, recurrence or onset of one or more symptoms associated with a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay.

As used herein, the term "purified" in the context of a compound, *e.g.*, a compound identified in accordance with the method of the invention, refers to a compound that is substantially free of chemical precursors or other chemicals when chemically synthesized. In a specific embodiment, the compound is 60%, preferably 65%, 70%, 75%, 80%, 85%, 90%, or 99% free of other, different compounds. In a preferred embodiment, a compound identified in accordance with the methods of the invention is purified.

As used herein, a "premature termination codon" or "premature stop codon" refers to the occurrence of a stop codon instead of a codon corresponding to an amino acid.

As used herein, a "reporter gene" refers to a gene by which modulation of premature translation termination and/or nonsense-mediated mRNA decay is ascertained.

In a preferred embodiment, the expression of a reporter gene is easily assayed and has an activity which is not normally found in the organism of which the translation extract is derived.

As used herein, the term "small molecule" and analogous terms include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (*i.e.*, including heterorganic and/or ganometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

As used herein, the terms "subject" and "patient" are used interchangeably herein. The terms "subject" and "subjects" refer to an animal, preferably a mammal including a non-primate (*e.g.*, a cow, pig, horse, cat, dog, rat, and mouse) and a primate (*e.g.*, a chimpanzee, a monkey such as a cynomolgous monkey and a human), and more preferably a human. In one embodiment, the subject is refractory or non-responsive to current therapies for a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay. In another embodiment, the subject is a farm animal (*e.g.*, a horse, a cow, a pig, etc.) or a pet (*e.g.*, a dog or a cat). In a preferred embodiment, the subject is a human.

As used herein, the term "synergistic" refers to a combination of a compound identified using one of the methods described herein, and another therapy (*e.g.*, a prophylactic or therapeutic agent), which combination is more effective than the additive effects of the therapies. A synergistic effect of a combination of therapies (*e.g.*, prophylactic or therapeutic agents) permits the use of lower dosages of one or more of the therapies and/or less frequent administration of said therapies to a subject with a proliferative disorder. The ability to utilize lower dosages of a therapy (*e.g.*, a prophylactic or therapeutic agent) and/or to administer said therapy less frequently reduces the toxicity associated with the administration of said therapy to a subject without reducing the efficacy of said therapies in the prevention, treatment, management or amelioration of a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay. In addition, a synergistic effect can result in improved efficacy of therapies (*e.g.*, agents) in the prevention, treatment, management or amelioration

of a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay. Finally, a synergistic effect of a combination of therapies (*e.g.*, prophylactic or therapeutic agents) may avoid or reduce adverse or unwanted side effects associated with the use of either therapy alone.

As used herein, the terms "therapeutic agent" and "therapeutic agents" refer to any agent(s) which can be used in the prevention, treatment, management or amelioration of one or more symptoms of a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay. In certain embodiments, the term "therapeutic agent" refers to a compound identified in the screening assays described herein. In other embodiments, the term "therapeutic agent" refers to an agent other than a compound identified in the screening assays described herein which is known to be useful for, or has been or is currently being used to prevent, treat, manage or ameliorate a proliferative disorder or one or more symptoms thereof.

As used herein, the term "therapeutically effective amount" refers to that amount of a therapy (*e.g.*, a therapeutic agent) sufficient to result in (i) the amelioration of one or more symptoms of a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay, (ii) prevent advancement of a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay, (iii) cause regression of av disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay, or (iv) to enhance or improve the therapeutic effect(s) of another therapy (*e.g.*, therapeutic agent).

As used herein, the terms "treat", "treatment" and "treating" refer to the reduction or amelioration of the progression, severity and/or duration of a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay or one or more symptoms thereof resulting from the administration of one or more compounds identified in accordance the methods of the invention, or a combination of one or more compounds identified in accordance with the invention and another therapy.

As used herein, the terms "therapy" and "therapies" refer to any method, protocol and/or agent that can be used in the prevention, treatment, management or amelioration of a disesase or disorder or one or more symptoms thereof. In certain embodiments, such terms refer to chemotherapy, radiation therapy, surgery, supportive therapy and/or other therapies useful in the prevention, treatment, management or

amelioration of a disease or disorder or one or more symptoms thereof known to skilled medical personnel.

4. BRIEF DESCRIPTION OF THE FIGURES

FIG. 1. Translation of a wild-type luciferase RNA in the *in vitro* translation reaction. Reaction mixtures were prepared containing varying amounts of wild-type luciferase RNA and varying amounts of HeLa cell extract. The amount of luciferase produced was monitored in a Turner luminometer by the addition of luciferase substrate (Promega).

FIG. 2. Translation of a nonsense containing (UGA) luciferase RNA in the *in vitro* translation reaction. Reaction mixtures were prepared containing varying amounts of luciferase RNA harboring a UGA nonsense mutation and varying amounts of HeLa cell extract. The amount of luciferase was monitored in a Turner luminometer by the addition of luciferase substrate (Promega).

FIG. 3. Translation of wild-type luciferase RNA by incubating the cells on ice prior to lysis. HeLa cell pellets were incubated on ice or not incubated on ice prior to lysis and the effect of the incubation on the translation activity of the cell-extract was measured in an *in vitro* translation reaction for luciferase production

FIG. 4 Translation of a nonsense (UGA) containing luciferase RNA in the *in vitro* translation reaction. Reaction mixtures were prepared with luciferase RNA containing a UGA nonsense mutation. Gentamicin was (GENT) or was not added (UNT) added to the reaction mixture and the amount of luciferase produced was monitored in a Viewlux luminometer by the addition of luciferase substrate (Promega).

FIG. 5. The amount of luciferase produced was monitored in a Viewlux luminometer by the addition of luciferase substrate (Promega).

FIG. 6A-6B. 6A: Nonsense suppression in cells harboring a luciferase nonsense allele. Stable cell lines harboring the UGA, UAA and UAG nonsense alleles of luciferase were treated overnight with Compound A, Compound B, and Gentamicin. The following day, the level of suppression was determined by measuring the amount of luminescence produced. The fold suppression above control cells treated with solvent was calculated and plotted vs.

concentration of compound. 6B: Nonsense suppression in cells harboring a luciferase nonsense allele. Stable cell lines harboring the UGA, UAA and UAG nonsense alleles of luciferase were treated overnight with Compound A, and gentamicin. The following day, the level of suppression was determined by measuring the amount of luminescence produced. The fold suppression above control cells treated with solvent was calculated and plotted vs. concentration of compound.

FIG. 7A-7B. Chemical footprinting analysis of Compound A on the human 28S rRNA. 100 pmol of ribosomes were incubated with 100 μM compound, followed by treatment with chemical modifying agents (dimethyl sulfate [DMS] and kethoxal [KE]). Following chemical modification, rRNA was prepared and analyzed in primer extension reactions using end-labeled oligonucleotides hybridizing to rRNA. Panel A (lanes 1-3 DMS modification; lanes 4-6 KE modification): Lanes 1 and 4, DMSO treated; 2 and 5, paromomycin treated; 3 and 6, Compound A treated; 4. A sequencing reaction (indicated by lanes GATC in panel A) was run in parallel as a marker.

FIG. 8. Functional CFTR expression monitored as cAMP-induced anion efflux using the halide-sensitive fluorophore 6-methoxy-N-(3-sulphopro-pyl) quinolinium (SPQ). Compound A increases cAMP-stimulated chloride channel activity in cells expressing the W1282X mutation. Cells were initially loaded in a hypotonic buffer containing SPQ and sodium iodide; iodide quenches SPQ fluorescence (Yang *et al.*, 1993, Hum Mol Genet. 2(8):1253-1261). Sodium iodide in the bath was replaced by sodium nitrate at 2 min; since nitrate does not interact with SPQ, fluorescence increased as cell iodide is lost to the bath. A cAMP stimulation cocktail (10 μM forskolin, 100 μM cpt-cAMP and 100 μM IBMX) was added at 6 min. Fluorescence was then quenched again by returning sodium iodide to the bath at 10 min. Functional CFTR expression was monitored as the dequenching of SPQ fluorescence caused by cAMP-induced iodide efflux.

FIG. 9. Immunohistochemistry of myotubes from primary cell culture from mdx muscle. The presence of dystrophin was detected by mAb to the COOH-terminus of dystrophin (F192A12) followed by a rhodamine-conjugated anti-mouse IgG. Dystrophin was present in mdx myotubes treated with 20 μM Compound A (left) and in mdx myotubes

treated with 200 μM gentamicin (center). Little dystrophin was detected in untreated mdx myotubes (right).

FIG. 10A-10F. Immunohistochemistry of muscle cross-sections to view dystrophin. C57 control tibialis anterior (TA) muscle displayed positive staining for dystrophin (panel D). Muscle cross-sections from mdx mice treated with gentamicin (200 μM, panel A) and Compound A (10 μM panel B; 20 μM panel C) displayed positive staining for dystrophin. Muscle from untreated mdx mice (panel E) or from cross sections not treated with primary antibody (panel F) show only minimal staining.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention provides methods for identifying compounds that modulate premature translation termination and/or nonsense-mediated mRNA decay. In particular, the invention provides simple, rapid and sensitive methods for identifying compounds that modulate premature translation termination and/or nonsense-mediated mRNA decay. Any gene encoding a premature stop codon can be used in the cell-based and cell-free assays described herein to identify compounds that modulate premature translation termination and/or nonsense-mediated mRNA decay. The cell-based and cell-free assays described herein can be utilized in a high throughput format to screen libraries of compounds to identify those compounds that modulate premature translation termination and/or nonsense-mediated mRNA decay.

Reporter gene-based assays can be utilized to identify a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay. The reporter gene-based assays described herein may be conducted by contacting a compound with a cell containing a nucleic acid sequence comprising a reporter gene, wherein said reporter gene comprises a premature stop codon, and measuring the expression and/or activity of the reporter gene. Alternatively, the reporter gene-based assays may be conducted by contacting a compound with a cell-free extract and a nucleic acid sequence comprising a reporter gene, wherein said reporter gene comprises a premature stop codon, and measuring the expression of said reporter gene. The reporter gene-based assays may also be conducted by: (a) contacting a compound with a cell containing a first nucleic acid sequence and a second nucleic acid sequence, wherein the first nucleic acid sequence comprises a reporter gene operably linked to a regulatory element and the second nucleic acid sequence comprises a nucleotide sequence encoding a regulatory protein or a subunit

thereof with a premature stop codon and the regulatory protein regulates the expression of the reporter gene; and (b) measuring the expression and/or activity of the reporter gene. Further, the reporter gene-based assays may be conducted by: (a) contacting a compound with a cell-free extract, a first nucleic acid sequence and a second nucleic acid sequence, wherein the first nucleic acid sequence comprises a reporter gene operably linked to a regulatory element and the second nucleic acid sequence comprises a nucleotide sequence encoding a regulatory protein or a subunit thereof with a premature stop codon and the regulatory protein regulates the expression of the reporter gene; and (b) measuring the expression and/or activity of the reporter gene. The alteration in reporter gene expression relative to a previously determined reference range, or the expression of the reporter gene in the absence of the compound or an appropriate control (*e.g.*, a negative control) in such reporter-gene based assays indicates that a particular compound modulates premature translation termination and/or nonsense-mediated mRNA decay.

The structure of the compounds identified in the assays described herein that modulate changes in post-transcriptional gene regulation can be determined utilizing assays well-known to one of skill in the art or described herein. The methods used will depend, in part, on the nature of the library screened. For example, assays or microarrays of compounds, each having an address or identifier, may be deconvoluted, *e.g.*, by cross-referencing the positive sample to an original compound list that was applied to the individual test assays. Alternatively, the structure of the compounds identified herein may be determined using mass spectrometry, nuclear magnetic resonance ("NMR"), X ray crystallography, or vibrational spectroscopy.

The invention encompasses the use of the compounds that modulate premature translation termination and/or nonsense-mediated mRNA decay that were identified in accordance with the methods described herein. In particular, the invention encompasses the use of compounds identified as lead compounds for the development of prophylactic or therapeutic agents in the prevention, treatment, management and/or amelioration of a disease associated with, characterized by or caused by a nonsense mutation. Such diseases include, but are not limited to, cystic fibrosis, muscular dystrophy, heart disease, cancer, retinitis pigmentosa, collagen disorders, Tay-Sachs disease, blood disorders, kidney stones, ataxia-telangiectasia, lysosomal storage diseases, and tuberous sclerosis.

Section 5.1 describes genes with premature translation stop codons and cells and cell-free extracts that are useful in the methods of the invention. Section 5.2 describes

libraries of compounds. Section 5.4 describes reporter gene-based assays for identifying compounds that modulate premature translation termination and/or nonsense-mediated mRNA decay. Section 5.5 describes naturally occurring genes with premature stop codons and examples of diseases associated with such genes. Section 5.6 describes secondary biological screens. Section 5.7 describes the methods for designing congeners or analogs of compounds identified in accordance with the methods of the invention. Section 5.8 describes uses of compounds identified in accordance with the methods of the invention for preventing, treating, managing or ameliorating a disease or abnormal condition in a subject associated with, characterized by or caused by a premature stop codon. Section 5.9 describes methods of administering compounds identified in accordance with the invention to a subject in need thereof.

5.1. Reporter Gene Constructs, Transfected Cells and Cell-Free Extracts

The invention provides for reporter genes to ascertain the effects of a compound on premature translation termination and/or nonsense-mediated mRNA decay. In general, the level of expression and/or activity of a reporter gene product is indicative of the effect of the compound on premature translation termination and/or nonsense-mediated mRNA decay.

The invention provides for specific vectors comprising a reporter gene operably linked to one or more regulatory elements and host cells transfected with the vectors. The invention also provides for the *in vitro* translation of a reporter gene flanked by one or more regulatory elements. A reporter gene may or may not contain a premature stop codon depending on the assay conducted. Techniques for practicing this specific aspect of this invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, and recombinant DNA manipulation and production, which are routinely practiced by one of skill in the art. See, *e.g.*, Sambrook, 1989, Molecular Cloning, A Laboratory Manual, Second Edition; DNA Cloning, Volumes I and II (Glover, Ed. 1985); Oligonucleotide Synthesis (Gait, Ed. 1984); Nucleic Acid Hybridization (Hames & Higgins, Eds. 1984); Transcription and Translation (Hames & Higgins, Eds. 1984); Animal Cell Culture (Freshney, Ed. 1986); Immobilized Cells and Enzymes (IRL Press, 1986); Perbal, A Practical Guide to Molecular Cloning (1984); Gene Transfer Vectors for Mammalian Cells (Miller & Calos, Eds. 1987, Cold Spring Harbor Laboratory); Methods in Enzymology, Volumes 154 and 155 (Wu & Grossman, and Wu, Eds., respectively), (Mayer & Walker, Eds., 1987); Immunochemical Methods in Cell and Molecular Biology (Academic Press, London, Scopes, 1987), Expression of Proteins in

Mammalian Cells Using Vaccinia Viral Vectors in Current Protocols in Molecular Biology, Volume 2 (Ausubel et al., Eds., 1991).

5.1.1. Reporter Genes

Any reporter gene well-known to one of skill in the art may be used in reporter gene constructs to ascertain the effect of a compound on premature translation termination. Reporter genes refer to a nucleotide sequence encoding a protein, polypeptide or peptide that is readily detectable either by its presence or activity. Reporter genes may be obtained and the nucleotide sequence of the elements determined by any method well-known to one of skill in the art. The nucleotide sequence of a reporter gene can be obtained, *e.g.*, from the literature or a database such as GenBank. Alternatively, a polynucleotide encoding a reporter gene may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular reporter gene is not available, but the sequence of the reporter gene is known, a nucleic acid encoding the reporter gene may be chemically synthesized or obtained from a suitable source (*e.g.*, a cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the reporter gene) by PCR amplification. Once the nucleotide sequence of a reporter gene is determined, the nucleotide sequence of the reporter gene may be manipulated using methods well-known in the art for the manipulation of nucleotide sequences, *e.g.*, recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel *et al.*, eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate reporter genes having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, a reporter gene is any naturally-occurring gene with a premature stop codon. Genes with premature stop codons that are useful in the present invention include, but are not limited to, the genes described below. In an alternative embodiment, a reporter gene is any gene that is not known in nature to contain a premature stop codon. Examples of reporter genes include, but are not limited to, luciferase (*e.g.*, firefly luciferase, renilla luciferase, and click beetle luciferase), green fluorescent protein ("GFP") (*e.g.*, green fluorescent protein, yellow fluorescent protein, red fluorescent protein, cyan fluorescent protein, and blue fluorescent protein), beta-galactosidase ("beta-gal"), beta-glucoronidase, beta-lactamase, chloramphenicol acetyltransferase ("CAT"), and

alkaline phosphatase ("AP"). Alternatively, a reporter gene can also be a protein tag, such as, but not limited to, myc, His, FLAG, or GST, so that nonsense suppression will produce the peptide and the protein can be monitored by an ELISA, a western blot, or any other immunoassay to detect the protein tag. Such methods are well known to one of skill in the art. In a preferred embodiment, the reporter gene is easily assayed and has an activity which is not normally found in the gene of interest. Table 1 below lists various reporter genes and the properties of the products of the reporter genes that can be assayed. In a preferred embodiment, a reporter gene utilized in the reporter constructs is easily assayed and has an activity which is not normally found in the cell or organism of interest.

TABLE 1: Reporter Genes and the Properties of the Reporter Gene Products

Reporter Gene	Protein Activity & Measurement
CAT (chloramphenicol acetyltransferase)	Transfers radioactive acetyl groups to chloramphenicol or detection by thin layer chromatography and autoradiography
GAL (beta-galactosidase)	Hydrolyzes colorless galactosides to yield colored products.
GUS (beta-glucuronidase)	Hydrolyzes colorless glucuronides to yield colored products.
LUC (luciferase)	Oxidizes luciferin, emitting photons
GFP (green fluorescent protein)	Fluorescent protein without substrate
SEAP (secreted alkaline phosphatase)	Luminescence reaction with suitable substrates or with substrates that generate chromophores
HRP (horseradish peroxidase)	In the presence of hydrogen oxide, oxidation of 3,3',5,5'-tetramethylbenzidine to form a colored complex
AP (alkaline phosphatase)	Luminescence reaction with suitable substrates or with substrates that generate chromophores

Described hereinbelow in further detailed are specific reporter genes and characteristics of those reporter genes.

5.1.1.1. Luciferase

Luciferases are enzymes that emit light in the presence of oxygen and a substrate (luciferin) and which have been used for real-time, low-light imaging of gene expression in cell cultures, individual cells, whole organisms, and transgenic organisms (reviewed by Greer & Szalay, 2002, Luminescence 17(1):43-74).

As used herein, the term "luciferase" is intended to embrace all luciferases, or recombinant enzymes derived from luciferases which have luciferase activity. The luciferase genes from fireflies have been well characterized, for example, from the *Photinus* and *Luciola* species (see, *e.g.*, International Patent Publication No. WO 95/25798 for *Photinus pyralis*, European Patent Application No. EP 0 524 448 for *Luciola cruciata* and *Luciola lateralis*, and Devine et al., 1993, Biochim. Biophys. Acta 1173(2):121-132 for *Luciola mingrelica)*. Other eucaryotic luciferase genes include, but are not limited to, the click beetle (*Photinus plagiophthalamus*, see, *e.g.*, Wood et al., 1989, Science 244:700-702), the sea panzy (*Renilla reniformis*, see, *e.g.*, Lorenz et al., 1991, Proc Natl Acad Sci U S A 88(10):4438-4442), and the glow worm (*Lampyris noctiluca*, see *e.g.*, Sula-Newby et al., 1996, Biochem J. 313:761-767). The click beetle is unusual in that different members of the species emit bioluminescence of different colors, which emit light at 546 nm (green), 560 nm (yellow-green), 578 nm (yellow) and 593 nm (orange) (see, *e.g.* U.S. Patent Nos. 6,475,719; 6,342,379; and 6,217,847, the disclosures of which are incorporated by reference in their entireties). Bacterial luciferin-luciferase systems include, but are not limited to, the bacterial lux genes of terrestrial *Photorhabdus luminescens* (see, *e.g.*, Manukhov et al., 2000, Genetika 36(3):322-30) and marine bacteria *Vibrio fischeri* and *Vibrio harveyi* (see, *e.g.*, Miyamoto et al., 1988, J Biol Chem. 263(26):13393-9, and Cohn et al., 1983, Proc Natl Acad Sci USA., 80(1):120-3, respectively). The luciferases encompassed by the present invention also includes the mutant luciferases described in U.S. Patent No. 6,265,177 to Squirrell *et al.*, which is hereby incorporated by reference in its entirety.

In a specific embodiment, the luciferase is a firefly luciferase, a renilla luciferase, or a click beetle luciferase, as described in any one of the references listed *supra*, the disclosures of which are incorporated by reference in their entireties.

5.1.1.2. Green Fluorescent Protein

Green fluorescent protein ("GFP") is a 238 amino acid protein with amino acid residues 65 to 67 involved in the formation of the chromophore which does not require additional substrates or cofactors to fluoresce (see, *e.g.*, Prasher et al., 1992, Gene 111:229-233; Yang et al., 1996, Nature Biotechnol. 14:1252-1256; and Cody et al., 1993, Biochemistry 32:1212-1218).

As used herein, the term "green fluorescent protein" or "GFP" is intended to embrace all GFPs (including the various forms of GFPs which exhibit colors other than green), or recombinant enzymes derived from GFPs which have GFP activity. In a preferred embodiment, GFP includes green fluorescent protein, yellow fluorescent protein,

red fluorescent protein, cyan fluorescent protein, and blue fluorescent protein. The native gene for GFP was cloned from the bioluminescent jellyfish *Aequorea victoria* (see, *e.g.*, Morin et al., 1972, J. Cell Physiol. 77:313-318). Wild type GFP has a major excitation peak at 395 nm and a minor excitation peak at 470 nm. The absorption peak at 470 nm allows the monitoring of GFP levels using standard fluorescein isothiocyanate (FITC) filter sets. Mutants of the GFP gene have been found useful to enhance expression and to modify excitation and fluorescence. For example, mutant GFPs with alanine, glycine, isoleucine, or threonine substituted for serine at position 65 result in mutant GFPs with shifts in excitation maxima and greater fluorescence than wild type protein when excited at 488 nm (see, *e.g.*, Heim et al., 1995, Nature 373:663-664; U.S. Patent No. 5,625,048; Delagrave et al., 1995, Biotechnology 13:151-154; Cormack et al., 1996, Gene 173:33-38; and Cramer et al., 1996, Nature Biotechnol. 14:315-319). The ability to excite GFP at 488 nm permits the use of GFP with standard fluorescence activated cell sorting ("FACS") equipment. In another embodiment, GFPs are isolated from organisms other than the jellyfish, such as, but not limited to, the sea pansy, *Renilla reriformis*.

Techniques for labeling cells with GFP in general are described in U.S. Patent Nos. 5,491,084 and 5,804,387, which are incorporated by reference in their entireties; Chalfie et al., 1994, Science 263:802-805; Heim et al., 1994, Proc. Natl. Acad. Sci. USA 91:12501-12504; Morise et al., 1974, Biochemistry 13:2656-2662; Ward et al., 1980, Photochem. Photobiol. 31:611-615; Rizzuto et al., 1995, Curr. Biology 5:635-642; and Kaether & Gerdes, 1995, FEBS Lett 369:267-271. The expression of GFPs in *E. coli* and *C. elegans* are described in U.S. Patent No. 6,251,384 to Tan et al., which is incorporated by reference in its entirety. The expression of GFP in plant cells is discussed in Hu & Cheng, 1995, FEBS Lett 369:331-33, and GFP expression in *Drosophila* is described in Davis et al., 1995, Dev. Biology 170:726-729.

5.1.1.3. Beta Galactosidase

Beta galactosidase ("beta-gal") is an enzyme that catalyzes the hydrolysis of beta-galactosides, including lactose, and the galactoside analogs o-nitrophenyl-beta-D-galactopyranoside ("ONPG") and chlorophenol red-beta-D-galactopyranoside ("CPRG") (see, *e.g.*, Nielsen et al., 1983 Proc Natl Acad Sci USA 80(17):5198-5202; Eustice et al., 1991, Biotechniques 11:739-742; and Henderson et al., 1986, Clin. Chem. 32:1637-1641). The beta-gal gene functions well as a reporter gene because the protein product is extremely stable, resistant to proteolytic degradation in cellular lysates, and easily assayed. When

ONPG is used as the substrate, beta-gal activity can be quantitated with a spectrophotometer or microplate reader.

As used herein, the term "beta galactosidase" or "beta-gal" is intended to embrace all beta-gals, including *lacZ* gene products, or recombinant enzymes derived from beta-gals which have beta-gal activity. The beta-gal gene functions well as a reporter gene because the protein product is extremely stable, resistant to proteolytic degradation in cellular lysates, and easily assayed. In an embodiment where ONPG is the substrate, beta-gal activity can be quantitated with a spectrophotometer or microplate reader to determine the amount of ONPG converted at 420 nm. In an embodiment when CPRG is the substrate, beta-gal activity can be quantitated with a spectrophotometer or microplate reader to determine the amount of CPRG converted at 570 to 595 nm. In yet another embodiment, the beta-gal activity can be visually ascertained by plating bacterial cells transformed with a beta-gal construct onto plates containing Xgal and IPTG. Bacterial colonies that are dark blue indicate the presence of high beta-gal activity and colonies that are varying shades of blue indicate varying levels of beta-gal activity.

5.1.1.4. Beta-Glucuronidase

Beta-glucuronidase ("GUS") catalyzes the hydrolysis of a very wide variety of beta-glucuronides, and, with much lower efficiency, hydrolyzes some beta-galacturonides. GUS is very stable, will tolerate many detergents and widely varying ionic conditions, has no cofactors, nor any ionic requirements, can be assayed at any physiological pH, with an optimum between 5.0 and 7.8, and is reasonably resistant to thermal inactivation (see, *e.g.*, U.S. Patent No. 5,268,463, which is incorporated by reference in its entirety).

In one embodiment, the GUS is derived from the *Esherichia coli* beta-glucuronidase gene. In alternate embodiments of the invention, the beta-glucuronidase encoding nucleic acid is homologous to the *E. coli* beta-glucuronidase gene and/or may be derived from another organism or species.

GUS activity can be assayed either by fluorescence or spectrometry, or any other method described in U.S. Patent No. 5,268,463, the disclosure of which is incorporated by reference in its entirety. For a fluorescent assay, 4-trifluoromethylumbelliferyl beta-D-glucuronide is a very sensitive substrate for GUS. The fluorescence maximum is close to 500 nm--bluish green, where very few plant compounds fluoresce or absorb. 4-trifluoromethylumbelliferyl beta-D-glucuronide also fluoresces much more strongly near neutral pH, allowing continuous assays to be performed more readily

than with MUG. 4-trifluoromethylumbelliferyl beta-D-glucuronide can be used as a fluorescent indicator *in vivo*. The spectrophotometric assay is very straightforward and moderately sensitive (Jefferson et al., 1986, Proc. Natl. Acad. Sci. USA 86:8447-8451). A preferred substrate for spectrophotometric measurement is p-nitrophenyl beta-D-glucuronide, which when cleaved by GUS releases the chromophore p-nitrophenol. At a pH greater than its pK_a (around 7.15) the ionized chromophore absorbs light at 400-420 nm, giving a yellow color.

5.1.1.5. Beta-Lactamases

Beta-lactamases are nearly optimal enzymes in respect to their almost diffusion-controlled catalysis of beta-lactam hydrolysis, making them suited to the task of an intracellular reporter enzyme (see, *e.g.*, Christensen et al., 1990, Biochem. J. 266: 853-861). They cleave the beta-lactam ring of beta-lactam antibiotics, such as penicillins and cephalosporins, generating new charged moieties in the process (see, *e.g.*, O'Callaghan et al., 1968, Antimicrob. Agents. Chemother. 8: 57-63 and Stratton, 1988, J. Antimicrob. Chemother. 22, Suppl. A: 23-35). A large number of beta-lactamases have been isolated and characterized, all of which would be suitable for use in accordance with the present invention (see, *e.g.*, Richmond & Sykes, 1978, Adv.Microb.Physiol. 9:31-88 and Ambler, 1980, Phil. Trans. R. Soc. Lond. [Ser.B.] 289: 321-331, the disclosures of which are incorporated by reference in their entireties).

The coding region of an exemplary beta-lactamase employed has been described in U.S. Patent No. 6,472,205, Kadonaga et al., 1984, J.Biol.Chem. 259: 2149-2154, and Sutcliffe, 1978, Proc. Natl. Acad. Sci. USA 75: 3737-3741, the disclosures of which re incorporated by reference in their entireties. As would be readily apparent to those skilled in the field, this and other comparable sequences for peptides having beta-lactamase activity would be equally suitable for use in accordance with the present invention. The combination of a fluorogenic substrate described in U.S. Patent Nos. 6,472,205, 5,955,604, and 5,741,657, the disclosures of which are incorporated by reference in their entireties, and a suitable beta-lactamase can be employed in a wide variety of different assay systems, such as are described in U.S. Patent No. 4,740,459, which is hereby incorporated by reference in its entirety.

5.1.1.6. Chloramphenicol Acetyltransferase

Chloramphenicol acetyl transferase ("CAT") is commonly used as a reporter gene in mammalian cell systems because mammalian cells do not have detectable levels of

CAT activity. The assay for CAT involves incubating cellular extracts with radiolabeled chloramphenicol and appropriate co-factors, separating the starting materials from the product by, for example, thin layer chromatography ("TLC"), followed by scintillation counting (see, *e.g.*, U.S. Patent No. 5,726,041, which is hereby incorporated by reference in its entirety).

As used herein, the term "chloramphenicol acetyltransferase" or "CAT" is intended to embrace all CATs, or recombinant enzymes derived from CAT which have CAT activity. While it is preferable that a reporter system which does not require cell processing, radioisotopes, and chromatographic separations would be more amenable to high through-put screening, CAT as a reporter gene may be preferable in situations when stability of the reporter gene is important. For example, the CAT reporter protein has an *in vivo* half life of about 50 hours, which is advantageous when an accumulative versus a dynamic change type of result is desired.

5.1.1.7. Secreted Alkaline Phosphatase

The secreted alkaline phosphatase ("SEAP") enzyme is a truncated form of alkaline phosphatase, in which the cleavage of the transmembrane domain of the protein allows it to be secreted from the cells into the surrounding media. In a preferred embodiment, the alkaline phosphatase is isolated from human placenta.

As used herein, the term "secreted alkaline phosphatase" or "SEAP" is intended to embrace all SEAP or recombinant enzymes derived from SEAP which have alkaline phosphatase activity. SEAP activity can be detected by a variety of methods including, but not limited to, measurement of catalysis of a fluorescent substrate, immunoprecipitation, HPLC, and radiometric detection. The luminescent method is preferred due to its increased sensitivity over calorimetric detection methods. The advantages of using SEAP is that a cell lysis step is not required since the SEAP protein is secreted out of the cell, which facilitates the automation of sampling and assay procedures. A cell-based assay using SEAP for use in cell-based assessment of inhibitors of the Hepatitis C virus protease is described in U.S. Patent No. 6,280,940 to Potts et al. which is hereby incorporated by reference in its entirety.

5.1.2. Proteins That Regulate the Expression of Reporter Genes

The invention provides a nucleic acid sequence comprising a nucleotide sequence encoding a regulatory protein or a component or subunit thereof, which regulatory protein binds to a regulatory element operably linked to a reporter gene and regulates the

expression of the reporter gene. The expression of the full-length regulatory protein or component or subunit thereof is suppressed or inhibited in the absence of a compound that suppresses premature translation termination and/or nonsense-mediated mRNA decay because of the presence of a premature stop codon or nonsense mutation within the open reading frame of the nucleotide sequence encoding the regulatory protein. The expression of the full-length regulatory protein or component or subunit thereof is, thus, contingent on the suppression of the premature stop codon or nonsense mutation by a compound. As the expression of the reporter gene is regulated by a regulatory element responsive to the full-length regulatory protein, reporter gene expression should only be detected in the presence of a compound that suppresses the premature stop codon or nonsense mutation.

The location of the premature stop codon or nonsense mutation is N-terminal to the native stop codon of the regulatory protein or component or subunit thereof. In a specific embodiment, the premature stop codon or nonsense mutation is at least 15 nucleotides, preferably at least 25 nucleotides, at least 50 nucleotides, at least 75 nucleotides or at least 100 nucleotides from the start codon in the open reading frame of the nucleotide sequence encoding the regulatory protein or a component or subunit thereof. In another embodiment, the premature stop codon or nonsense mutation is at least 15 nucleotides, preferably at least 25 nucleotides, at least 50 nucleotides, at leat 75 nucleotides or at least 100 nucleotides from the native stop codon in the open reading frame of the nucleotide sequence encoding the regulatory protein or a component or subunit thereof. In another embodiment, the premature stop codon in the open reading frame of the nucleotide sequences. In another embodiment, the premature stop codon in the open reading frame of the nucleotide sequence encoding the regulatory protein or a component or subunit thereof is in the context of UGAA, UGAC, UGAG, UGAU, UAGA, UAGC, UAGG, UAGU, UAAA, UAAC, UAAG or UAAU. In yet another embodiment, the nucleotide sequence encoding the regulatory protein or a component or subunit thereof, contains or is engineered to contain two, three, four or more stop codons. In another embodiment, the premature stop codon in the open reading frame of the nucleotide sequences encoding the regulatory protein or a component or subunit thereof is UAG or UGA.

In one embodiment, the invention provides a nucleic acid sequence comprising a nucleotide sequence encoding a regulatory protein with a premature stop codon or nonsense mutation. In accordance with this embodiment, the nucleic acid sequence can encode a naturally-occurring gene with a premature stop codon or nonsense mutation or the nucleic acid sequence can be engineered to contain a premature stop codon

or nonsense mutation using techniques well-known in the art. In this case, the expression of the full-length regulatory protein regulates the expression of the reporter gene which is detected by techniques well-known in the art or described herein.

In another embodiment, the invention provides a first nucleic acid sequence and a second nucleic acid sequence, wherein the first nucleic acid sequence comprises a nucleotide sequence encoding a first fusion protein comprising (or alternatively, consisting of) a DNA binding domain and a first protein, and the second nucleic acid sequence comprises a nucleotide sequence encoding a second fusion protein comprising (or alternatively, consisting of) an activation domain and a second protein. In accordance with this embodiment, the nucleotide sequence encoding the first or second fusion protein may contain or be engineered to contain a premature stop codon or nonsense mutation. The first fusion protein and second fusion protein interact and produce a regulatory protein when the premature stop codon or nonsense mutation is suppressed by a compound. Thus, the production of a functional regulatory protein is dependent on suppression of a premature stop codon or nonsense mutation. In this case, the production of the functional regulatory protein regulates the expression of the reporter gene which is detected by techniques well-known in the art or described herein.

In one embodiment of the invention, the protein that regulates expression of a gene contains domains which are associated with various activities related to transcriptional regulation, including, but not limited to, binding and activation. In one embodiment, a binding domain of a regulatory protein is one that recognizes and specifically associates with a sequence of at least two nucleotides of a nucleic acid. Nucleic acids that can be recognized by a binding domain of a protein, include, but are not limited to, DNA and RNA both single and multiple stranded. In a more specific embodiment, a binding domain can adopt one of a number of conformations or motifs, known in the art, including but not limited to, zinc finger, leucine zipper, helix turn helix and helix loop helix. In a more preferred embodiment, the binding domain protein is one that specifically recognizes a region of a nucleic acid. Such recognition can occur through a number of interactions, including, but not limited to, covalent, hyrophobic and van der Waals. In another embodiment, an activation domain is one that modulates, regulates, enhances, suppresses or controls the expression of a gene. In such an embodiment, the activation domain can modulate, regulate, enhance, suppress or control the expression of a gene by interacting, either directly or indirectly, with other compounds or proteins that are required or involved in gene expression. In one embodiment, such domains can be expressed as

proteins that are fused with other proteins suitable for the described assays. For example, in one embodiment, the activation domain of a regulatory protein is expressed as part of a protein or polypeptide encoded by a nucleic acid, and the binding domain of a regulatory protein is expressed as a part of a protein or polypeptide encoded by another nucleic acid. In a more specific embodiment, such binding and regulatory domains are expressed as fusion proteins with other proteins with properties that are suitable to the assay. In an example of an embodiment suitable to the described assays, the binding and regulatory domains are expressed on separate fusion proteins with proteins that interact with each other. For example, the binding domain can be expressed as a chimeric protein that is fused to another protein known to associate with another protein that is expressed from a separate nucleic acid and fused to the activation domain. In such an embodiment, a regulatory complex is formed by the association between the binding domain and the activation domain expressed as parts of the described fusion proteins. Interaction between the two domains can be mediated or initiated by a number of means, preferably through inter or intra molecular associations between the parts of the described fusion proteins that are known to interact with one another. Examples of proteins or complexes that contain domains that bind to nucleic acids in addition to possesing regulatory functions include, but are not limited to, GAL4, glucocorticoid and estrogen receptors (GR and ER), Xfin protein, GCN4, and the transcription factor Max in complex with oncogene Myc.

The invention relates to the identification of compounds that modulate premature translation termination or nonsense-mediated mRNA decay, using, in some instances, a reporter based assay. The invention provides for the identification of compounds that modulated premature translation termination via a nonsense stop codon in a nucleic acids. Such nucleic acids include, but are not limited to, DNA and RNA. In a more certain embodiment, the nucleic acid is RNA. In another embodiment, the nucleic acid is single stranded. In other embodiments, the nucleic acids are single stranded. In yet other embodiments, the nucleic acids are more than single stranded, e.g., double, triple or quadruple stranded.

5.1.3. Stop Codons

The present invention provides for methods for screening and identifying compounds that modulate premature translation termination and/or nonsense-mediated mRNA decay. A reporter gene may be engineered to contain a premature stop codon or may naturally contain a premature stop codon. Alternatively, a protein, polypeptide or peptide that regulates (directly or indirectly) the expression of a reporter gene may be

engineered to contain or may naturally contain a premature stop codon. The premature stop codon may any one of the stop codons known in the art including UAG, UAA and UGA.

The stop codons are UAG, UAA, and UGA, *i.e.*, signals to the ribosome to terminate protein synthesis, presumably through protein release factors. Even though the use of these stop codons is widespread, they are not universal. For example, UGA specifies tryptophan in the mitochondria of mammals, yeast, Neurospora crassa, Drosophila, protozoa, and plants (see, *e.g.*, Breitenberger & RajBhandary, 1985, Trends Biochem Sci 10:481). Other examples include the use of UGA for tryptophan in Mycoplasma and, in ciliated protozoa, the use of UAA and UAG for glutamine (see, *e.g.*, Jukes et al., 1987, Cold Spring Harb Symp Quant Biol. 52:769-776), the use of UGA for cysteine in the ciliate Euplotes aediculatus (see, *e.g.*, Kervestin et al., 2001, EMBO Rep 2001 Aug;2(8):680-684), the use of UGA for tryptophan in Blepharisma americanum and the use of UAR for glutamine in Tetrahymena, and three spirotrichs, Stylonychia lemnae, S. mytilus, and Oxytricha trifallax (see, *e.g.*, Lozupone et al., 2001, Curr Biol 11(2):65-74). It has been proposed that the ancestral mitochondrion was bearing the universal genetic code and subsequently reassigned the UGA codon to tryptophan independently, at least in the lineage of ciliates, kinetoplastids, rhodophytes, prymnesiophytes, and fungi (see, *e.g.*, Inagaki et al., 1998, J Mol Evol 47(4):378-384).

The readthrough of stop codons also occurs in positive-sense ssRNA viruses by a variety of naturally occurring suppressor tRNAs. Such naturally-occurring suppressor tRNAs include, but are not limited to, cytoplasmic tRNATyr, which reads through the UAG stop codon; cytoplasmic tRNAsGln, which read through UAG and UAA; cytoplasmic tRNAsLeu, which read through UAG; chloroplast and cytoplasmic tRNAsTrp, which read through UGA; chloroplast and cytoplasmic tRNAsCys, which read through UGA; cytoplasmic tRNAsArg, which read through UGA (see, *e.g.*, Beier & Grimm, 2001, Nucl Acids Res 29(23):4767-4782 for a review); and the use of selenocysteine to suppress UGA in E. coli (see, e.g., Baron & Böck, 1995, The selenocysteine inserting tRNA species: structure and function. In Söll,D. and RajBhandary,U.L. (eds), tRNA: Structure, Biosynthesis and Function, ASM Press, Washington, DC, pp. 529 544). The mechanism is thought to involve unconventional base interactions and/or codon context effects.

As described above, the stop codons are not necessarily universal, with consideration variation amongst organelles (*e.g.*, mitochondria and chloroplasts), viruses (*e.g.*, single strand viruses), and protozoa (*e.g.*, ciliated protozoa) as to whether the codons UAG, UAA, and UGA signal translation termination or encode amino acids. Even though a

single release factor most probably recognizes all of the stop codons in eucaryotes, it appears that all of the stop codons are not suppressed in a similar matter. For example, in the yeast *Saccharomyces pombe*, nonsense suppression has to be strictly codon specific (see, *e.g.*, Hottinger et al., 1984, EMBO J 3:423-428). In another example, significant differences were found in the degree of suppression amongst three UAG codons and two UAA codons in different mRNA contexts in *Escherichia coli* and in human 293 cells, although data suggested that the context effects of nonsense suppression operated differently in *E. coli* and human cells (see, *e.g.*, Martin et al., 1989, Mol Gen Genet 217(2 3):411 8). Since unconventional base interactions and/or codon context effects have been implicated in nonsense suppression, it is conceivable that compounds involved in nonsense suppression of one stop codon may not necessarily be involved in nonsense suppression of another stop codon. In other words, compounds involved in suppressing UAG codons may not necessarily be involved in suppressing UGA codons.

In a specific embodiment, a reporter gene or a gene encoding a protein, polypeptide or peptide that regulates the expression of a reporter gene contains or is engineered to contain the premature stop codon UAG. In another embodiment, a reporter gene or a gene encoding a protein, polypeptide or peptide that regulates the expression of a reporter gene contains or is engineered to contain the premature stop codon UGA. In yet another embodiment, a reporter gene or a gene encoding a protein, polypeptide or peptide that regulates the expression of a reporter gene contains or is engineered to contain a premature stop codon in the context of UGAA, UGAC, UGAG, UGAU, UAGA, UAGC, UAGG, UAGU, UAAA, UAAC, UAAG or UAAU.

In a particular embodiment, a reporter gene or a gene encoding a protein, polypeptide or peptide that regulates the expression of a reporter gene contains or is engineered to contain two, three, four or more stop codons. In accordance with this embodiment, the stop codons are preferably at least 10 nucleotides, at least 15 nucleotides, at least 20 nucleotides, at least 25 nucleotides, at least 30 nucleotides, at least 35 nucleotides, at least 40 nucleotides, at least 45 nucleotides, at least 50 nucleotides, at least 75 nucleotides or at least 100 nucleotides apart from each other. Further, in accordance with this embodiment, at least one of the stop codons is preferably UAG or UGA.

In a specific embodiment, a reporter gene or a gene encoding a protein, polypeptide or peptide that regulates the expression of a reporter gene contains or is engineered to contain a premature stop codon at least 15 nucleotides, preferably at least 20 nucleotides, at least 25 nucleotides, at least 30 nucleotides, at least 35 nucleotides, at least

40 nucleotides, at least 45 nucleotides, at least 50 nucleotides or at least 75 nucleotides from the start codon in the coding sequence. In another embodiment, a reporter gene or a gene encoding a protein, polypeptide or peptide that regulates the expression of a reporter gene contains or is engineered to contain a premature stop codon at least 15 nucleotides, preferably at least 25 nucleotides, at least 50 nucleotides, at least 75 nucleotides, at least 100 nucleotides, at least 125 nucleotides, at least 150, at least 175 nucleotides or at least 200 nucleotides from the native stop codon in the coding sequence of the full-length reporter gene product or protein, polypeptide or peptide. In another embodiment, a reporter gene or a gene encoding a protein, polypeptide or peptide that regulates the expression of a reporter gene contains or is engineered to contain a premature stop codon at least 15 nucleotides (preferably at least 20 nucleotides, at least 25 nucleotides, at least 30 nucleotides, at least 35 nucleotides, at least 40 nucleotides, at least 45 nucleotides, at least 50 nucleotides or at least 75 nucleotides) from the start codon in the coding sequence and at least 15 nucleotides (preferably at least 25 nucleotides, at least 50 nucleotides, at least 75 nucleotides, at least 100 nucleotides, at least 125 nucleotides, at least 150, at least 175 nucleotides or at least 200 nucleotides) from the native stop codon in the coding sequence of the full-length reporter gene product or protein, polypeptide or peptide. In accordance with these embodiments, the premature stop codon is preferably UAG or UGA.

The premature translation stop codon can be produced by *in vitro* mutagenesis techniques such as, but not limited to, polymerase chain reaction ("PCR"), linker insertion, oligonucleotide-mediated mutagenesis, and random chemical mutagenesis.

5.1.4. Vectors

The nucleotide sequence encoding for a protein, polypeptide or peptide (*e.g.*, a reporter gene, or a protein, polypeptide or peptide that regulates the expression of a reporter gene) can be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. The necessary transcriptional and translational elements can also be supplied by the protein, polypeptide or peptide. The regulatory regions and enhancer elements can be of a variety of origins, both natural and synthetic. In a specific embodiment, a reporter gene is operably linked to regulatory element that is responsive to a regulatory protein whose expression is dependent upon the suppression of a premature stop codon.

A variety of host-vector systems may be utilized to express a protein, polypeptide or peptide. These include, but are not limited to, mammalian cell systems

infected with virus (*e.g.*, vaccinia virus, adenovirus, *etc.*); insect cell systems infected with virus (*e.g.*, baculovirus); microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA; and stable cell lines generated by transformation using a selectable marker. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

Any of the methods previously described for the insertion of DNA fragments into a vector may be used to construct expression vectors containing a chimeric nucleic acid consisting of appropriate transcriptional/translational control signals and the protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of a first nucleic acid sequence encoding a protein, polypeptide or peptide, such as a reporter gene, may be regulated by a second nucleic acid sequence so that the first nucleic acid sequence is expressed in a host transformed with the second nucleic acid sequence. For example, expression of a nucleic acid sequence encoding a protein, polypeptide or peptide, such as a reporter gene, may be controlled by any promoter/enhancer element known in the art, such as a constitutive promoter, a tissue-specific promoter, or an inducible promoter. Specific examples of promoters which may be used to control gene expression include, but are not limited to, the SV40 early promoter region (Bernoist & Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Kamaroff et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), or the *tac* promoter (DeBoer et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25); see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; plant expression vectors comprising the nopaline synthetase promoter region (Herrera-Estrella et al., Nature 303:209-213) or the cauliflower mosaic virus 35S RNA promoter (Gardner, et al., 1981, Nucl. Acids Res. 9:2871), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella et al., 1984, Nature 310:115-120); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals:

elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58; alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94; myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314:283-286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378).

In a specific embodiment, a vector is used that comprises a promoter operably linked to a reporter gene, one or more origins of replication, and, optionally, one or more selectable markers (*e.g.*, an antibiotic resistance gene). In a preferred embodiment, the vectors are CMV vectors, T7 vectors, lac vectors, pCEP4 vectors, 5.0/F vectors, or vectors with a tetracycline-regulated promoter (*e.g.*, pcDNA™5/FRT/TO from Invitrogen). Some vectors may be obtained commercially. Non-limiting examples of useful vectors are described in Appendix 5 of Current Protocols in Molecular Biology, 1988, ed. Ausubel et al., Greene Publish. Assoc. & Wiley Interscience, which is incorporated herein by reference; and the catalogs of commercial suppliers such as Clontech Laboratories, Stratagene Inc., and Invitrogen, Inc.

Expression vectors containing a construct of the present invention can be identified by the following general approaches: (a) nucleic acid hybridization, (b) presence or absence of "marker" nucleic acid functions, (c) expression of inserted sequences, and (d) sequencing. In the first approach, the presence of a particular nucleic acid sequence inserted in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to the inserted nucleic acid sequence. In the

second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" nucleic acid functions (*e.g.*, thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, *etc.*) caused by the insertion of the nucleic acid sequence of interest in the vector. For example, if the nucleic acid sequence of interest is inserted within the marker nucleic acid sequence of the vector, recombinants containing the insert can be identified by the absence of the marker nucleic acid function. In the third approach, recombinant expression vectors can be identified by assaying the product expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of the particular nucleic acid sequence.

In a preferred embodiment, nucleic acid sequences encoding proteins, polypeptides or peptides are cloned into stable cell line expression vectors. In a preferred embodiment, the stable cell line expression vector contains a site specific genomic integration site. In another preferred embodiment, the reporter gene construct is cloned into an episomal mammalian expression vector.

5.1.5. Transfection

Once a vector encoding the appropriate gene has been synthesized, a host cell is transformed or transfected with the vector of interest. The use of stable transformants is preferred. In a preferred embodiment, the host cell is a mammalian cell. In a more preferred embodiment, the host cell is a human cell. In another embodiment, the host cells are primary cells isolated from a tissue or other biological sample of interest. Host cells that can be used in the methods of the present invention include, but are not limited to, hybridomas, pre-B cells, 293 cells, 293T cells, HeLa cells, HepG2 cells, K562 cells, 3T3 cells. In another preferred embodiment, the host cells are derived from tissue specific to the nucleic acid sequence encoding a protein, polypeptide or peptide. In another preferred embodiment, the host cells are immortalized cell lines derived from a source, *e.g.*, a tissue. Other host cells that can be used in the present invention include, but are not limited to, bacterial cells, yeast cells, virally-infected cells, or plant cells.

Preferred mammalian host cells include but are not limited to those derived from humans, monkeys and rodents, (see, for example, Kriegler M. in "Gene Transfer and Expression: A Laboratory Manual", New York, Freeman & Co. 1990), such as monkey kidney cell line transformed by SV40 (COS-7, ATCC Accession No. CRL 1651); human embryonic kidney cell lines (293, 293-EBNA, or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen. Virol., 36:59, 1977; baby hamster kidney cells

(BHK, ATCC Accession No. CCL 10); chinese hamster ovary-cells-DHFR (CHO, Urlaub and Chasin. Proc. Natl. Acad. Sci. 77; 4216, 1980); mouse sertoli cells (Mather, Biol. Reprod. 23:243-251, 1980); mouse fibroblast cells (NIH-3T3), monkey kidney cells (CVI ATCC Accession No. CCL 70); african green monkey kidney cells (VERO-76, ATCC Accession No. CRL-1587); human cervical carcinoma cells (HELA, ATCC Accession No. CCL 2); canine kidney cells (MDCK, ATCC Accession No. CCL 34); buffalo rat liver cells (BRL 3A, ATCC Accession No. CRL 1442); human lung cells (W138, ATCC Accession No. CCL 75); human liver cells (Hep G2, HB 8065); and mouse mammary tumor cells (MMT 060562, ATCC Accession No. CCL51).

Other useful eukaryotic host-vector system may include yeast and insect systems. In yeast, a number of vectors containing constitutive or inducible promoters may be used with *Saccharomyces cerevisiae* (baker's yeast), *Schizosaccharomyces pombe* (fission yeast), *Pichia pastoris,* and *Hansenula polymorpha* (methylotropic yeasts). For a review see, Current Protocols in Molecular Biology, Vol. 2, 1988, Ed. Ausubel et al., Greene Publish. Assoc. & Wiley Interscience, Ch. 13; Grant et al., 1987, Expression and Secretion Vectors for Yeast, in Methods in Enzymology, Eds. Wu & Grossman, 1987, Acad. Press, N.Y., Vol. 153, pp. 516-544; Glover, 1986, DNA Cloning, Vol. II, IRL Press, Wash., D.C., Ch. 3; and Bitter, 1987, Heterologous Gene Expression in Yeast, Methods in Enzymology, Eds. Berger & Kimmel, Acad. Press, N.Y., Vol. 152, pp. 673-684; and The Molecular Biology of the Yeast *Saccharomyces*, 1982, Eds. Strathern et al., Cold Spring Harbor Press, Vols. I and II.

Standard methods of introducing a nucleic acid sequence of interest into host cells can be used. Transformation may be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus and transducing a host cell with the virus, and by direct uptake of the polynucleotide. The transformation procedure used depends upon the host to be transformed. Mammalian transformations (*i.e.,* transfections) by direct uptake may be conducted using the calcium phosphate precipitation method of Graham & Van der Eb, 1978, Virol. 52:546, or the various known modifications thereof. Other methods for introducing recombinant polynucleotides into cells, particularly into mammalian cells, include dextran-mediated transfection, calcium phosphate mediated transfection, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the polynucleotides into nuclei. Such methods are well-known to one of skill in the art.

In a preferred embodiment, stable cell lines containing the constructs of interest are generated for high throughput screening. Such stable cells lines may be generated by introducing a construct comprising a selectable marker, allowing the cells to grow for 1-2 days in an enriched medium, and then growing the cells on a selective medium. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, et al., 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1962, Proc. Natl. Acad. Sci. USA 48:2026), and adenine phosphoribosyltransferase (Lowy, et al., 1980, Cell 22:817) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, anti-metabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, et al., 1980, Natl. Acad. Sci. USA 77:3567; O'Hare, et al., 1981, Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, et al., 1981, J. Mol. Biol. 150:1); and hygro, which confers resistance to hygromycin (Santerre, et al., 1984, Gene 30:147) genes.

5.1.6. Cell-Free Extracts

The invention provides for the translation of a nucleic acid sequence encoding a protein, polypeptide or peptide (with or without a premature translation stop codon) in a cell-free system. Techniques for practicing the specific aspect of this invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, and recombinant DNA manipulation and production, which are routinely practiced by one of skill in the art. See, *e.g.*, Sambrook, 1989, Molecular Cloning, A Laboratory Manual, Second Edition; DNA Cloning, Volumes I and II (Glover, Ed. 1985); and Transcription and Translation (Hames & Higgins, Eds. 1984).

Any technique well-known to one of skill in the art may be used to generate cell-free extracts for translation. For example, the cell-free extracts can be generated by centrifuging cells and clarifying the supernatant. In one embodiment, the cells are incubated on ice during the preparation of the cell-free extract. In another embodiment, the cells are incubated on ice at least 12 hours, at least 24 hours, at least two days, at least five days, at least one week, at least longer than one week. In a more specific embodiment, the

cells are incubated on ice at least long enough so as to improve the translation activity of the cell extract in comparison to cell extracts that are not incubated on ice. In yet another embodiment, the cells are incubated at a temperature between about 0 °C and 10 °C. In a preferred embodiment, the cells are incubated at about 4 °C.

In another preferred embodiment, the cells are centrifuged at a low speed to isolate the cell-free extract for *in vitro* translation reactions. In a preferred embodiment, the cell extract is the supernatant from cells that are centrifuged at about 2 x g to 20,000 x g. In a more preferred embodiment, the cell extract is the supernatant from cells that are centrifuged at about 5 x g to 15,000 x g. In an even more preferred embodiment, the cell extract is the supernatant from cells that are centrifuged at about 10,000 x g. Alternatively, in a preferred embodiment, the cell-free extract is about the S1 to S50 extract. In a more preferred embodiment, the cell extract is about the S5 to S25 extract. In an even more preferred embodiment, the cell extract is about the S10 extract.

The cell-free translation extract may be isolated from cells of any species origin. In another embodiment, the cell-free translation extract is isolated from yeast, cultured mouse or rat cells, Chinese hamster ovary (CHO) cells, *Xenopus* oocytes, reticulocytes, wheat germ, or rye embryo (see, *e.g.*, Krieg & Melton, 1984, Nature 308:203 and Dignam *et al.*, 1990 Methods Enzymol. 182:194-203). Alternatively, the cell-free translation extract, *e.g.*, rabbit reticulocyte lysates and wheat germ extract, can be purchased from, *e.g.*, Promega, (Madison, WI). In another embodiment, the cell-free translation extract is prepared as described in International Patent Publication No. WO 01/44516 and U.S. Patent No. 4,668,625 to Roberts, the disclosures of which are incorporated by reference in their entireties. In a preferred embodiment, the cell-free extract is an extract isolated from human cells. In a more preferred embodiment, the human cells are HeLa cells. It is preferred that the endogenous expression of the genes with the premature translation codons is minimal, and preferably absent, in the cells from which the cell-free translation extract is prepared.

Systems for the *in vitro* transcription of RNAs with the gene of interest cloned in an expression vectors using promoters such as, but not limited to, Sp6, T3, or T7 promoters (see, *e.g.*, expression vectors from Invitrogen, Carlesbad, CA; Promega, Madison, WI; and Stratagene, La Jolla, CA), and the subsequent transcription of the gene with the appropriate polymerase are well-known to one of skill in the art (see, *e.g.*, Contreras *et al.*, 1982, Nucl. Acids. Res. 10:6353). In another embodiment, the gene encoding the premature stop codon can be PCR-amplified with the appropriate primers,

with the sequence of a promoter, such as but not limited to, Sp6, T3, or T7 promoters, incorporated into the upstream primer, so that the resulting amplified PCR product can be *in vitro* transcribed with the appropriate polymerase.

Alternatively, a coupled transcription-translation system can be used for the expression of a gene encoding a premature stop codon in a cell free extract, such as the TnT® Coupled Transcription/Translation System (Promega, Madison, WI) or the system described in U.S. Patent No. 5,895,753 to Mierendorf *et al.*, which is incorporated by reference in its entirety.

5.2. Compounds

Libraries screened using the methods of the present invention can comprise a variety of types of compounds. Examples of libraries that can be screened in accordance with the methods of the invention include, but are not limited to, peptoids; random biooligomers; diversomers such as hydantoins, benzodiazepines and dipeptides; vinylogous polypeptides; nonpeptidal peptidomimetics; oligocarbamates; peptidyl phosphonates; peptide nucleic acid libraries; antibody libraries; carbohydrate libraries; and small molecule libraries (preferably, small organic molecule libraries). In some embodiments, the compounds in the libraries screened are nucleic acid or peptide molecules. In a non-limiting example, peptide molecules can exist in a phage display library. In other embodiments, the types of compounds include, but are not limited to, peptide analogs including peptides comprising non-naturally occurring amino acids, *e.g.*, D-amino acids, phosphorous analogs of amino acids, such as α-amino phosphoric acids and α-amino phosphoric acids, or amino acids having non-peptide linkages, nucleic acid analogs such as phosphorothioates and PNAs, hormones, antigens, synthetic or naturally occurring drugs, opiates, dopamine, serotonin, catecholamines, thrombin, acetylcholine, prostaglandins, organic molecules, pheromones, adenosine, sucrose, glucose, lactose and galactose. Libraries of polypeptides or proteins can also be used in the assays of the invention. In some embodiments, the compounds are nucleic acid or peptide molecules. In a non-limiting example, peptide molecules can exist in a phage display library.

In a preferred embodiment, the combinatorial libraries are small organic molecule libraries, such as, but not limited to, benzodiazepines, isoprenoids, thiazolidinones, metathiazanones, pyrrolidines, morpholino compounds, and benzodiazepines. In another embodiment, the combinatorial libraries comprise peptoids; random bio-oligomers; benzodiazepines; diversomers such as hydantoins, benzodiazepines and dipeptides;, vinylogous polypeptides; nonpeptidal peptidomimetics; oligocarbamates;

peptidyl phosphonates; peptide nucleic acid libraries; antibody libraries; or carbohydrate libraries. Combinatorial libraries are themselves commercially available (see, *e.g.*, ComGenex, Princeton, New Jersey; Asinex, Moscow, Ru, Tripos, Inc., St. Louis, Missouri; ChemStar, Ltd, Moscow, Russia; 3D Pharmaceuticals, Exton, Pennsylvania; Martek Biosciences, Columbia, Maryland; *etc.*).

In a preferred embodiment, the library is preselected so that the compounds of the library are more amenable for cellular uptake. For example, compounds are selected based on specific parameters such as, but not limited to, size, lipophilicity, hydrophilicity, and hydrogen bonding, which enhance the likelihood of compounds getting into the cells. In another embodiment, the compounds are analyzed by three-dimensional or four-dimensional computer computation programs.

In one embodiment, the combinatorial compound library for the methods of the present invention may be synthesized. There is a great interest in synthetic methods directed toward the creation of large collections of small organic compounds, or libraries, which could be screened for pharmacological, biological or other activity. The synthetic methods applied to create vast combinatorial libraries are performed in solution or in the solid phase, *i.e.*, on a solid support. Solid-phase synthesis makes it easier to conduct multi-step reactions and to drive reactions to completion with high yields because excess reagents can be easily added and washed away after each reaction step. Solid-phase combinatorial synthesis also tends to improve isolation, purification and screening. However, the more traditional solution phase chemistry supports a wider variety of organic reactions than solid-phase chemistry.

Combinatorial compound libraries of the present invention may be synthesized using the apparatus described in U.S. Patent No. 6,190,619 to Kilcoin *et al.*, which is hereby incorporated by reference in its entirety. U.S. Patent No. 6,190,619 discloses a synthesis apparatus capable of holding a plurality of reaction vessels for parallel synthesis of multiple discrete compounds or for combinatorial libraries of compounds.

In one embodiment, the combinatorial compound library can be synthesized in solution. The method disclosed in U.S. Patent No. 6,194,612 to Boger *et al.*, which is hereby incorporated by reference in its entirety, features compounds useful as templates for solution phase synthesis of combinatorial libraries. The template is designed to permit reaction products to be easily purified from unreacted reactants using liquid/liquid or solid/liquid extractions. The compounds produced by combinatorial synthesis using the template will preferably be small organic molecules. Some compounds in the library may

mimic the effects of non-peptides or peptides. In contrast to solid phase synthesize of combinatorial compound libraries, liquid phase synthesis does not require the use of specialized protocols for monitoring the individual steps of a multistep solid phase synthesis (Egner *et al.*, 1995, J.Org. Chem. 60:2652; Anderson *et al.*, 1995, J. Org. Chem. 60:2650; Fitch *et al.*, 1994, J. Org. Chem. 59:7955; Look *et al.*, 1994, J. Org. Chem. 49:7588; Metzger *et al.*, 1993, Angew. Chem., Int. Ed. Engl. 32:894; Youngquist *et al.*, 1994, Rapid Commun. Mass Spect. 8:77; Chu *et al.*, 1995, J. Am. Chem. Soc. 117:5419; Brummel *et al.*, 1994, Science 264:399; Stevanovic *et al.*, 1993, Bioorg. Med. Chem. Lett. 3:431).

Combinatorial compound libraries useful for the methods of the present invention can be synthesized on solid supports. In one embodiment, a split synthesis method, a protocol of separating and mixing solid supports during the synthesis, is used to synthesize a library of compounds on solid supports (see *e.g.*, Lam *et al.*, 1997, Chem. Rev. 97:41-448; Ohlmeyer *et al.*, 1993, Proc. Natl. Acad. Sci. USA 90:10922-10926 and references cited therein). Each solid support in the final library has substantially one type of compound attached to its surface. Other methods for synthesizing combinatorial libraries on solid supports, wherein one product is attached to each support, will be known to those of skill in the art (*see, e.g.*, Nefzi *et al.*, 1997, Chem. Rev. 97:449-472).

As used herein, the term "solid support" is not limited to a specific type of solid support. Rather a large number of supports are available and are known to one skilled in the art. Solid supports include silica gels, resins, derivatized plastic films, glass beads, cotton, plastic beads, polystyrene beads, alumina gels, and polysaccharides. A suitable solid support may be selected on the basis of desired end use and suitability for various synthetic protocols. For example, for peptide synthesis, a solid support can be a resin such as p-methylbenzhydrylamine (pMBHA) resin (Peptides International, Louisville, KY), polystyrenes (*e.g.*, PAM-resin obtained from Bachem Inc., Peninsula Laboratories, etc.), including chloromethylpolystyrene, hydroxymethylpolystyrene and aminomethylpolystyrene, poly (dimethylacrylamide)-grafted styrene co-divinyl-benzene (*e.g.*, POLYHIPE resin, obtained from Aminotech, Canada), polyamide resin (obtained from Peninsula Laboratories), polystyrene resin grafted with polyethylene glycol (*e.g.*, TENTAGEL or ARGOGEL, Bayer, Tubingen, Germany) polydimethylacrylamide resin (obtained from Milligen/Biosearch, California), or Sepharose (Pharmacia, Sweden).

In some embodiments of the present invention, compounds can be attached to solid supports via linkers. Linkers can be integral and part of the solid support, or they may be nonintegral that are either synthesized on the solid support or attached thereto after

synthesis. Linkers are useful not only for providing points of compound attachment to the solid support, but also for allowing different groups of molecules to be cleaved from the solid support under different conditions, depending on the nature of the linker. For example, linkers can be, *inter alia*, electrophilically cleaved, nucleophilically cleaved, photocleavable, enzymatically cleaved, cleaved by metals, cleaved under reductive conditions or cleaved under oxidative conditions. In a preferred embodiment, the compounds are cleaved from the solid support prior to high throughput screening of the compounds.

In certain embodiments of the invention, the compound is a small molecule.

5.3. Reporter Gene-Based Screening Assays

Various *in vitro* assays can be used to identify and verify the ability of a compound to modulate premature translation termination and/or nonsense-mediated mRNA decay. Multiple *in vitro* assays can be performed simultaneously or sequentially to assess the affect of a compound on premature translation termination and/or nonsense-mediated mRNA decay. In a preferred embodiment, the *in vitro* assays described herein are performed in a high throughput format (*e.g.*, in microtiter plates).

5.3.1. Cell-Based Assays

After a vector containing the reporter gene construct and/or a vector(s) containing a nucleic acid sequence comprising a regulatory protein, a component or a subunit thereof is transformed or transfected into a host cell and a compound library is synthesized or purchased or both, the cells are used to screen the library to identify compounds that modulate premature translation termination and/or nonsense-mediated mRNA decay. The reporter gene-based assays may be conducted by contacting a compound or a member of a library of compounds with a cell (*e.g.*, a genetically engineered cell) containing a reporter gene construct comprising a reporter gene containing within the open reading frame of the reporter gene a premature stop codon or nonsense mutation; and measuring the expression and/or activity of the reporter gene. The reporter gene cell-based assays may also be conducted by: (a) contacting a compound with a cell containing a first nucleic acid sequence and a second nucleic acid sequence, wherein the first nucleic acid sequence comprises a regulatory element operably linked to a reporter gene and the second nucleic acid sequence comprises a nucleotide sequence with a premature stop codon or nonsense mutation that encodes a regulatory protein that binds to the regulatory element of

the first nucleic acid sequence and regulates the expression of the reporter gene; and (b) measuring the expression and/or activity of the reporter gene.

The reporter gene cell-based assays may also be conducted by: (a) contacting a compound with a cell containing a first nucleic acid sequence, a second nucleic acid sequence and a third nucleic acid sequence, wherein (i) the first nucleic acid sequence comprises a nucleotide sequence encoding a first fusion protein comprising a DNA binding domain and a first protein, the nucleotide sequence of the DNA binding domain or the first protein containing a premature stop codon or nonsense mutation, (ii) the second nucleic acid sequence comprises a nucleotide sequence encoding a second fusion protein comprising an activation domain and a second protein, the second protein interacting with the first protein to produce a regulatory protein, and (iii) the third nucleic acid sequence comprises a regulatory element operably linked to a reporter gene, the expression of the reporter gene being regulated by the binding of the regulatory protein to the regulatory element: and (b) measuring the expression and/or activity of the reporter gene. Further, the reporter gene cell-based assays may also be conducted by: (a) contacting a compound with a cell containing a first nucleic acid sequence, a second nucleic acid sequence and a third nucleic acid sequence, wherein (i) the first nucleic acid sequence comprises a nucleotide sequence encoding a first fusion protein comprising a DNA binding domain and a first protein, (ii) the second nucleic acid sequence comprises a nucleotide sequence encoding a second fusion protein comprising an activation domain and a second protein, the nucleotide sequence of the activation domain or the second protein containing a premature stop codon, and the second protein interacting with the first protein to produce a premature stop codon or nonsense mutation, and (iii) the third nucleic acid sequence comprises a regulatory element operably linked to a reporter gene, the expression of the reporter gene being regulated by the binding of the regulatory protein to the regulatory element: and (b) measuring the expression and/or activity of the reporter gene.

The alteration in reporter gene expression and/or activity in the reporter gene cell-based assays relative to a previously determined reference range, or to the expression or activity of the reporter gene in the absence of the compound or the presence of an appropriate control (*e.g.*, a negative control such as phosphate buffered saline) indicates that a particular compound modulates premature translation termination and/or nonsense-mediated mRNA decay. In particular, a decrease in reporter gene expression or activity relative to a previously determined reference range, or to the expression in the absence of the compound or the presence of an appropriate control (*e.g.*, a negative control) may,

depending upon the parameters of the reporter gene assay, indicate that a particular compound reduces or suppresses premature translation termination and/or nonsense-mediated mRNA decay. In contrast, an increase in reporter gene expression or activity relative to a previously determined reference range, or to the expression in the absence of the compound or the presence of an appropriate control (*e.g.*, a negative control) may, depending upon the parameters of the reporter gene-based assay, indicate that a particular compound enhances premature translation termination and/or nonsense-mediated mRNA decay.

The step of contacting a compound or a member of a library of compounds with cell in the reporter gene-based assays described herein is preferably conducted under physiologic conditions. In specific embodiment, a compound or a member of a library of compounds is added to the cells in the presence of an aqueous solution. In accordance with this embodiment, the aqueous solution may comprise a buffer and a combination of salts, preferably approximating or mimicking physiologic conditions. Alternatively, the aqueous solution may comprise a buffer, a combination of salts, and a detergent or a surfactant. Examples of salts which may be used in the aqueous solution include, but not limited to, KCl, NaCl, and/or $MgCl_2$. The optimal concentration of each salt used in the aqueous solution is dependent on the cells and compounds used and can be determined using routine experimentation. The step of contacting a compound or a member of a library of compounds with a cell containing a reporter gene construct and in some circumstances, a nucleic acid sequence encoding a regulatory protein, may be performed for at least 0.2 hours, 0.25 hours, 0.5 hours, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 8 hours, 10 hours, 12 hours, 18 hours, at least 1 day, at least 2 days or at least 3 days.

The expression of a reporter gene and/or activity of the protein encoded by the reporter gene in the cell-based reporter-gene assays may be detected by any technique well-known to one of skill in the art. The expression of a reporter gene can be readily detected, *e.g.*, by quantifying the protein and/or RNA encoded by said gene. Compounds that modulate premature translation termination and/or nonsense-mediated mRNA decay may be identified by changes in the gene encoding the premature translation stop codon, *i.e.*, there is readthrough of the premature translation stop codon and a longer gene product is detected. If a gene encoding a naturally-occurring premature translation stop codon is used, a longer gene product in the presence of a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay can be detected by any

method in the art that permits the detection of the longer polypeptide, such as, but not limited to, immunological methods.

Many methods standard in the art can be thus employed, including, but not limited to, immunoassays to detect and/or visualize gene expression (*e.g.*, Western blot, immunoprecipitation followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), immunocytochemistry, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, or an epitope tag using an antibody that is specific to the polypeptide encoded by the gene of interest) and/or hybridization assays to detect gene expression by detecting and/or visualizing respectively mRNA encoding a gene (*e.g.*, Northern assays, dot blots, *in situ* hybridization, etc), etc. Preferably, the antibody is specific to the C-terminal portion of the polypeptide used in an immunoassay. Such assays are routine and well known in the art (see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (*e.g.*, EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody which recognizes the antigen to the cell lysate, incubating for a period of time (*e.g.*, 1 to 4 hours) at 40° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 40° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody to immunoprecipitate a particular antigen can be assessed by, *e.g.*, western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (*e.g.*, pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (*e.g.*, 8%- 20% SDS-PAGE

depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (*e.g.*, PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (*e.g.*, PBS-Tween 20), blocking the membrane with primary antibody (the antibody which recognizes the antigen) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, *e.g.*, an anti-human antibody) conjugated to an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) or radioactive molecule (*e.g.*, ^{32}P or ^{125}I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding a primary antibody (which recognizes the antigen) conjugated to a detectable compound such as an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the primary antibody) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

Methods for detecting the activity of a protein encoded by a reporter gene will vary with the reporter gene used. Assays for the various reporter genes are well-known to one of skill in the art. For example, as described in Section 5.1.1., luciferase, beta-galactosidase ("beta-gal"), beta-glucoronidase ("GUS"), beta-lactamase, chloramphenicol acetyltransferase ("CAT"), and alkaline phosphatase ("AP") are enzymes that can be analyzed in the presence of a substrate and could be amenable to high throughput screening.

For example, the reaction products of luciferase, beta-galactosidase ("beta-gal"), and alkaline phosphatase ("AP") are assayed by changes in light imaging (*e.g.*, luciferase), spectrophotometric absorbance (*e.g.*, beta-gal), or fluorescence (*e.g.*, AP). Assays for changes in light output, absorbance, and/or fluorescence are easily adapted for high throughput screening. For example, beta-gal activity can be measured with a microplate reader. Green fluorescent protein ("GFP") activity can be measured by changes in fluorescence. For example, in the case of mutant GFPs that fluoresce at 488 nm, standard fluorescence activated cell sorting ("FACS") equipment can be used to separate cells based upon GFP activity.

Changes in mRNA stability of the gene encoding the premature translation stop codon can be measured. As discussed above, nonsense-mediated mRNA decay alters the stability of an mRNA with a premature translation stop codon so that such mRNA is targeted for rapid decay instead of translation. In the presence of a compound that modulates premature translation termination and/or nonsense-mediated mRNA decay, the stability of the mRNA with the premature translation stop codon is likely altered, *i.e.*, stabilized. Methods of measuring changes in steady state levels of mRNA are well-known to one of skill in the art. Such methods include, but are not limited to, Northern blots, dot blots, solution hybridization, RNase protection assays, and S1 nuclease protection assays, wherein the steady state levels of the mRNA of interest are measured with an appropriately labeled nucleic acid probe. Alternatively, methods such as semi-quantitative polymerase chain reaction ("PCR") can be used to measure changes in steady state levels of the mRNA of interest using the appropriate primers for amplification.

Alterations in the expression of a reporter gene may be determined by comparing the level of expression and/or activity of the reporter gene to a negative control (*e.g.*, PBS or another agent that is known to have no effect on the expression of the reporter gene) and optionally, a positive control (*e.g.*, an agent that is known to have an effect on the expression of the reporter gene, preferably an agent that effects premature translation termination and/or nonsense-mediated mRNA decay). Alternatively, alterations in the expression and/or activity of a reporter gene may be determined by comparing the level of expression and/or activity of the reporter gene to a previously determined reference range.

5.3.2. Cell-Free Extracts

After a vector containing the reporter gene construct and/or a vector(s) containing a nucleic acid sequence comprising a regulatory protein, a component or a subunit thereof is produced, a cell-free translation extract is generated or purchased, and a

compound library is synthesized or purchased or both, the cell-free translation extract and nucleic acid sequences are used to screen the library to identify compounds that modulate premature translation termination and/or nonsense-mediated mRNA decay. The reporter gene-based assays may be conducted in a cell-free manner by contacting a compound with a cell-free extract and a reporter gene construct comprising a reporter gene containing within the open reading frame of the reporter gene a premature stop codon or nonsense mutation, and measuring the expression and/or activity of said reporter gene. The reporter gene cell-free assays may also be conducted by contacting a compound with a cell-free extract and an *in vitro* transcribed RNA of a reporter gene, wherein the RNA product contains a premature stop codon or a nonsense mutation and measuring the expression and or activity of the protein encoded by the RNA product. Techniques for in vitro transcription are well-known to one of skill in the art or described herein (see, *e.g.* the Example in section 7). The reporter gene cell-free assays may also be conducted by: (a) contacting a compound with a cell-free extract, a first nucleic acid sequence and a second nucleic acid sequence, wherein the first nucleic acid sequence comprises a regulatory element operably linked to a reporter gene and the second nucleic acid sequence comprises a nucleotide sequence with a premature stop codon or nonsense mutation that encodes a regulatory protein that binds to the regulatory element of the first nucleic acid sequence and regulates the expression of the reporter gene; and (b) measuring the expression and/or activity of the reporter gene.

The reporter gene cell-free assays may also be conducted by: (a) contacting a compound with a cell-free extract, a first nucleic acid sequence, a second nucleic acid sequence and a third nucleic acid sequence, wherein (i) the first nucleic acid sequence comprises a nucleotide sequence encoding a first fusion protein comprising a DNA binding domain and a first protein, the nucleotide sequence of the DNA binding domain or the first protein containing a premature stop codon or nonsense mutation, (ii) the second nucleic acid sequence comprises a nucleotide sequence encoding a second fusion protein comprising an activation domain and a second protein, the second protein interacting with the first protein to produce a regulatory protein, and (iii) the third nucleic acid sequence comprises a regulatory element operably linked to a reporter gene, the expression of the reporter gene being regulated by the binding of the regulatory protein to the regulatory element: and (b) measuring the expression and/or activity of the reporter gene. Further, the reporter gene cell-free assays may also be conducted by: (a) contacting a compound with a cell-free extract, a first nucleic acid sequence, a second nucleic acid sequence and a third nucleic acid sequence, wherein (i) the first nucleic acid sequence comprises a nucleotide sequence

encoding a first fusion protein comprising a DNA binding domain and a first protein, (ii) the second nucleic acid sequence comprises a nucleotide sequence encoding a second fusion protein comprising an activation domain and a second protein, the nucleotide sequence of the activation domain or the second protein containing a premature stop codon or nonsense mutation, and the second protein interacting with the first protein to produce a regulatory protein, and (iii) the third nucleic acid sequence comprises a regulatory element operably linked to a reporter gene, the expression of the reporter gene being regulated by the binding of the regulatory protein to the regulatory element: and (b) measuring the expression and/or activity of the reporter gene.

In the cell-free reporter gene assays described herein, the alteration in reporter gene expression or activity relative to a previously determined reference range, or to the expression or activity of the reporter gene in the absence of the compound or the presence of an appropriate control (*e.g.*, a negative control) indicates that a particular compound modulates premature translation termination and/or nonsense-mediated mRNA decay. In particular, a decrease in reporter gene expression or activity relative to a previously determined reference range, or to the expression in the absence of the compound or the presence of an appropriate control (*e.g.*, a negative control) may, depending upon the parameters of the reporter gene assay, indicate that a particular compound reduces or suppresses premature translation termination and/or nonsense-mediated mRNA decay. In contrast, an increase in reporter gene expression or activity relative to a previously determined reference range, or to the expression in the absence of the compound or the presence of an appropriate control (*e.g.*, a negative control) may, depending upon the parameters of the reporter gene-based assay, indicate that a particular compound enhances premature translation termination and/or nonsense-mediated mRNA decay.

In accordance with the invention, the step of contacting a compound with a cell-free extract and a nucleic acid sequence in the reporter gene-based assays described herein is preferably conducted in an aqueous solution comprising a buffer and a combination of salts (such as KCl, NaCl and/or $MgCl_2$). The optimal concentration of each salt used in the aqueous solution is dependent on, *e.g.*, the protein, polypeptide or peptide encoded by the nucleic acid sequence (*e.g.*, the regulatory protein) and the compounds used, and can be determined using routine experimentation. In a specific embodiment, the aqueous solution approximates or mimics physiologic conditions. In another specific embodiment, the aqueous solution further comprises a detergent or a surfactant.

The cell-free reporter gene assays of the present invention can be performed using different incubation times. The cell-free extract and the nucleic acid sequence(s) (*e.g.*, a reporter gene) can be incubated together before the addition of a compound or a member of a library of compounds. In certain embodiments, the cell-free extract are incubated with a nucleic acid sequence(s) (*e.g.*, a reporter gene) before the addition of a compound or a member of a library of compounds for at least 0.2 hours, 0.25 hours, 0.5 hours, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 8 hours, 10 hours, 12 hours, 18 hours, or at least 1 day. In other embodiments, the cell-free extract, or the nucleic acid sequence(s) (*e.g.*, a reporter gene) is incubated with a compound or a member of a library of compounds before the addition of the nucleic acid sequence(s) (*e.g.*, a reporter gene), or the cell-free extract, respectively. In certain embodiments, a compound or a member of a library of compounds is incubated with a nucleic acid sequence(s) (*e.g.*, a reporter gene) or cell-free extract before the addition of the remaining component, *i.e.*, cell-free extract, or a nucleic acid sequence(s) (*e.g.*, a reporter gene), respectively, for at least 0.2 hours, 0.25 hours, 0.5 hours, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 8 hours, 10 hours, 12 hours, 18 hours, or at least 1 day. Once the reaction vessel comprises the components, *i.e.*, a compound or a member of a library of compounds, the cell-free extract and the nucleic acid sequence(s) (*e.g.*, a reporter gene), the reaction may be further incubated for at least 0.2 hours, 0.25 hours, 0.5 hours, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 8 hours, 10 hours, 12 hours, 18 hours, or at least 1 day.

The progress of the reaction in the cell-free reporter gene-based assays can be measured continuously. Alternatively, time-points may be taken at different times of the reaction to monitor the progress of the reaction in the cell-free reporter gene-based assays.

The activity of a compound in the cell-free extract can be determined by assaying the activity of a reporter protein encoded by a reporter gene, or alternatively, by quantifying the expression of the reporter gene by, for example, labeling the *in vitro* translated protein (*e.g.*, with ^{35}S-labeled methionine), northern blot analysis, RT-PCR or by immunological methods, such as western blot analysis or immunoprecipitation. Such methods are well-known to one of skill in the art. Examples of assays which can be used to measure the expression and/or activity of a reporter gene are described in Section 5.3.1 *supra*.

5.4. Characterization of the Structure of Compounds

If the library comprises arrays or microarrays of compounds, wherein each compound has an address or identifier, the compound can be deconvoluted, *e.g.*, by cross-

referencing the positive sample to original compound list that was applied to the individual test assays.

If the library is a peptide or nucleic acid library, the sequence of the compound can be determined by direct sequencing of the peptide or nucleic acid. Such methods are well known to one of skill in the art.

A number of physico-chemical techniques can be used for the *de novo* characterization of compounds that mediate premature translation termination and/or nonsense-mediated mRNA decay.

5.4.1. Mass Spectrometry

The invention provides, in part, for mass spectrometry methods to identify or characterize the compounds of the invention. Any mass spectrometric method can be used, for example, those employing an ionizer, ion analyzer and detector.

A number of techniques can be used in order to ionize a sample for invetigative or characterization purposes. Such techniques form the charged particles required for analysis. Examples of ionization methods include, but are not limited to, electron impact, chemical ionization, electrospray ionization, fast atom bombardment and matrix assisted laser desorption ionization. The technique used for ionization will depend on the type of analyte being examined and the conditions necessary for acquisition. For example, electron impact and chemical ionization would be preferred with a relatively small volatile sample with a mass of 1 to 1000 daltons; electrospray ionzation would be preferred with peptides, proteins and non-volatile samples with a mass of up to 200,000 daltons, fast atom bombardment would be preferred with carbohydrates, organometallics, peptides and nonvolatile compounds and matrix assisted laser desorption ionization would be preferred when examining peptides, proteins and nucleotides.

A number of ion analysis techniques can be used, in particular those where molecular ions and fragment ions are accelerated by manipulation of charged particles through the mass spectrometer. Such analyzers include, but are not limited to, quadropole, sector (magnetic and/or electrostatic), time of flight (TOF), and ion cyclotron resonance (ICR). The technique used for analysis would depend on the sample and the conditions for acquisition. For example, one might prefer quadropole when desiring a unit mass resolution, fast scan time, and low cost; one might prefer a sector analyzer when desiring high resolution and an exact mass; one might prefer time of flight when desiring no limitation for m/z maximum and a high throughput; and one might prefer ion cyclotron

resonance when desiring very high resolution an exact mass and also to perform ion chemistry.

Any ionizer method can be combined with any ion analyzer technique. There are many types of detectors that may be used as part of the mass spectrophotometric methods of the invention, in particular those that produce an electronic signal when struck by an ion. Calibration would be necessarily performed by introducing a well known compound into the instrument and adjusting the circuits so that the compound's molecular ion and fragment ions are reported accurately.

Mass spectrometry (*e.g.*, electrospray ionization ("ESI") and matrix-assisted laser desorption-ionization ("MALDI"), Fourier-transform ion cyclotron resonance ("FT-ICR") can be used both for high-throughput screening of compounds that bind to a target RNA and elucidating the structure of the compound.

MALDI uses a pulsed laser for desorption of the ions and a time-of-flight analyzer, and has been used for the detection of noncovalent tRNA:amino-acyl-tRNA synthetase complexes (Gruic-Sovulj *et al.*, 1997, J. Biol. Chem. 272:32084-32091). However, covalent cross-linking between the target nucleic acid and the compound is required for detection, since a non-covalently bound complex may dissociate during the MALDI process.

ESI mass spectrometry ("ESI-MS") has been of greater utility for studying non-covalent molecular interactions because, unlike the MALDI process, ESI-MS generates molecular ions with little to no fragmentation (Xavier *et al.*, 2000, Trends Biotechnol. 18(8):349-356). ESI-MS has been used to study the complexes formed by HIV Tat peptide and protein with the TAR RNA (Sannes-Lowery *et al.*, 1997, Anal. Chem. 69:5130-5135).

Fourier-transform ion cyclotron resonance ("FT-ICR") mass spectrometry provides high-resolution spectra, isotope-resolved precursor ion selection, and accurate mass assignments (Xavier *et al.*, 2000, Trends Biotechnol. 18(8):349-356). FT-ICR has been used to study the interaction of aminoglycoside antibiotics with cognate and non-cognate RNAs (Hofstadler *et al.*, 1999, Anal. Chem. 71:3436-3440; Griffey *et al.*, 1999, Proc. Natl. Acad. Sci. USA 96:10129-10133). As true for all of the mass spectrometry methods discussed herein, FT-ICR does not require labeling of the target RNA or a compound.

An advantage of mass spectroscopy is not only the elucidation of the structure of a compound, but also the determination of the structure of the compound bound

to a target RNA. Such information can enable the discovery of a consensus structure of a compound that specifically binds to a target RNA.

5.4.2. NMR Spectroscopy

The invention provides, in part, NMR spectroscopic techniques that may be used, for example, to characterize and identify small and large molecules of the invention. NMR methods are advantageous in understanding characteristics of the compounds of the invention because it allows rapid acquisition of single and multi-dimensional structural data about a compound in solution. Moreover, the NMR technique is a non-destructive technique that also provides dynamic information relating to a compound's behavior in complex or in association with other molecules of interest. There are a variety of techniques that can be used to examine compounds of the invention using NMR methods. In particular, any type of NMR spectrometer can be used, including, but not limited to, those of low, medium and high magnetic field. In a preferred embodiment, the NMR spectrometer that is used has a high magnetic field, in particular, if the compound has a high molecular weight, such as, those greater than 1000 daltons.

Any technique known in the art can be used to acquire data on the compounds and also to produce spectra for interpretation, including, but not limited to, those that measure through bond correlations and through space correlations. Both single and multi-dimensional spectra can be produced. In another embodiment, the technique that is used is homonuclear. In yet another embodiment, the technique is heteronuclear. In one embodiment of the invention, correlation spectroscopy, *e.g.*, COSY or TOCSY, methods are used to measure through bond correlations. In another embodiment of the invention, nuclear overhauser effect spectroscopy methods, *e.g.*, NOESY, are used to measure through space correlations. In yet another embodiment of the invention, multi-dimensional methods are used to identify relationships between heterologous nucleii, *e.g.*, heteronuclear single quantum coherence (HSQC) and heteronuclear multiple quantum coherence (HMQC).

In another embodiment of the invention, NMR methods are used to characterize compounds that are associated with other molecules. For example, complexed target nucleic acids can be examined by qualitatively determining changes in chemical shift, specifically from distances measured using relaxation effects, and NMR-based approaches have been used in the identification of small molecule binders of protein drug targets (Xavier *et al.*, 2000, Trends Biotechnol. 18(8):349-356). The determination of structure-activity relationships ("SAR") by NMR is the first method for NMR described in which small molecules that bind adjacent subsites are identified by two-dimentional ^{1}H-^{15}N spectra

of the target protein (Shuker *et al.*, 1996, Science 274:1531-1534). The signal from the bound molecule is monitored by employing line broadening, transferred NOEs and pulsed field gradient diffusion measurements (Moore, 1999, Curr. Opin. Biotechnol. 10:54-58). A strategy for lead generation by NMR using a library of small molecules has been recently described (Fejzo *et al.*, 1999, Chem. Biol. 6:755-769).

Other examples of NMR methods that can be used for the invention include, but are not limited to, one-dimensional, two-dimensional, three dimension, four dimensional NMR methods as well as correlation spectroscopy ("COSY"), and nuclear Overhauser effect ("NOE") spectroscopy. Such methods of structure determination of compounds are well known to one of skill in the art.

Similar to mass spectroscopy, an advantage of NMR is the not only the elucidation of the structure of a compound, but also the determination of the structure of the compound bound to the target RNA. Such information can enable the discovery of a consensus structure of a compound that specifically binds to a target RNA.

5.4.3. X-ray Crystallography

X-ray crystallography can be used to elucidate the structure of a compound. For a review of x-ray crystallography see, *e.g.*, Blundell et al. 2002, Nat Rev Drug Discov 1(1):45-54. The first step in x-ray crystallography is the formation of crystals. The formation of crystals begins with the preparation of highly purified and soluble samples. The conditions for crystallization is then determined by optimizing several solution variables known to induce nucleation, such as pH, ionic strength, temperature, and specific concentrations of organic additives, salts and detergent. Techniques for automating the crystallization process have been developed to automate the production of high-quality protein crystals. Once crystals have been formed, the crystals are harvested and prepared for data collection. The crystals are then analyzed by diffraction (such as multi-circle diffractometers, high-speed CCD detectors, and detector off-set). Generally, multiple crystals must be screened for structure determinations.

A number of methods can be used to acquire a diffraction patter so that a compound can be characterized. In one embodiment, an X-ray source is provided, for example, by a rotating anode generator producing an X-ray beam of a characteristic wavelength. There are a number of sources of X-ray radiation that may be used in the methods of the invention, including low and high intensity radiation. In one example, the tunable X-ray radiation is produced by a Synchrotron. In another embodiment, the primary X-ray beam is monochromated by either crystal monochromators or focusing mirrors and

the beam is passed through a helium flushed collimator. In a preferred embodiment, the crystal is mounted on a pin on a goniometer head, that is mounted to a goniometer which allows to position the crystal in different orientations in the beam. The diffracted X-rays can be recorded using a number of techniques, including, but not limited to image plates, multiwire detectors or CCD cameras. In other embodiments, flash cooling, for example, of protein crystals, to cryogenic temperatures (~100 K) offers many advantages, the most significant of which is the elimination of radiation damage.

5.4.4. Vibrational Spectrscopy

Vibrational spectroscopy (*e.g.*, but not limited to, infrared (IR) spectroscopy or Raman spectroscopy) can be used for elucidating the structure of a compound.

Infrared spectroscopy measures the frequencies of infrared light (wavelengths from 100 to 10,000 nm) absorbed by the compound as a result of excitation of vibrational modes according to quantum mechanical selection rules which require that absorption of light cause a change in the electric dipole moment of the molecule. The infrared spectrum of any molecule is a unique pattern of absorption wavelengths of varying intensity that can be considered as a molecular fingerprint to identify or characterize any compound.

Infrared spectra can be measured in a scanning mode by measuring the absorption of individual frequencies of light, produced by a grating which separates frequencies from a mixed-frequency infrared light source, by the compound relative to a standard intensity (double-beam instrument) or pre-measured ('blank') intensity (single-beam instrument). In a preferred embodiment, infrared spectra are measured in a pulsed mode ("FT-IR") where a mixed beam, produced by an interferometer, of all infrared light frequencies is passed through or reflected off the compound. The resulting interferogram, which may or may not be added with the resulting interferograms from subsequent pulses to increase the signal strength while averaging random noise in the electronic signal, is mathematically transformed into a spectrum using Fourier Transform or Fast Fourier Transform algorithms.

Raman spectroscopy measures the difference in frequency due to absorption of infrared frequencies of scattered visible or ultraviolet light relative to the incident beam. The incident monochromatic light beam, usually a single laser frequency, is not truly absorbed by the compound but interacts with the electric field transiently. Most of the light scattered off the sample will be unchanged (Rayleigh scattering) but a portion of the scatter light will have frequencies that are the sum or difference of the incident and molecular

vibrational frequencies. The selection rules for Raman (inelastic) scattering require a change in polarizability of the molecule. While some vibrational transitions are observable in both infrared and Raman spectrometry, must are observable only with one or the other technique. The Raman spectrum of any molecule is a unique pattern of absorption wavelengths of varying intensity that can be considered as a molecular fingerprint to identify any compound.

Raman spectra are measured by submitting monochromatic light to the sample, either passed through or preferably reflected off, filtering the Rayleigh scattered light, and detecting the frequency of the Raman scattered light. An improved Raman spectrometer is described in US Patent No. 5,786,893 to Fink et al., which is hereby incorporated by reference.

Vibrational microscopy can be measured in a spatially resolved fashion to address single beads by integration of a visible microscope and spectrometer. A microscopic infrared spectrometer is described in U.S. Patent No. 5,581,085 to Reffner et al., which is hereby incorporated by reference in its entirety. An instrument that simultaneously performs a microscopic infrared and microscopic Raman analysis on a sample is described in U.S. Patent No. 5,841,139 to Sostek et al., which is hereby incorporated by reference in its entirety.

In one embodiment of the method, compounds are synthesized on polystyrene beads doped with chemically modified styrene monomers such that each resulting bead has a characteristic pattern of absorption lines in the vibrational (IR or Raman) spectrum, by methods including but not limited to those described by Fenniri et al., 2000, J. Am. Chem. Soc. 123:8151-8152. Using methods of split-pool synthesis familiar to one of skill in the art, the library of compounds is prepared so that the spectroscopic pattern of the bead identifies one of the components of the compound on the bead. Beads that have been separated according to their ability to bind target RNA can be identified by their vibrational spectrum. In one embodiment of the method, appropriate sorting and binning of the beads during synthesis then allows identification of one or more further components of the compound on any one bead. In another embodiment of the method, partial identification of the compound on a bead is possible through use of the spectroscopic pattern of the bead with or without the aid of further sorting during synthesis, followed by partial resynthesis of the possible compounds aided by doped beads and appropriate sorting during synthesis.

In another embodiment, the IR or Raman spectra of compounds are examined while the compound is still on a bead, preferably, or after cleavage from bead,

using methods including but not limited to photochemical, acid, or heat treatment. The compound can be identified by comparison of the IR or Raman spectral pattern to spectra previously acquired for each compound in the combinatorial library.

5.5. Naturally Occurring Genes with Premature Stop Codons: Examples of Disorders and Diseases

The invention provides for naturally occurring genes with premature stop codons to ascertain the effects of compounds on premature translation termination and/or nonsense-mediated mRNA decay. In general, the expression of the gene product, in particular, a full-length gene product, is indicative of the effect of the compounds on premature translation termination and/or nonsense-mediated mRNA decay.

In a preferred embodiment, the naturally occurring genes with premature stop codons are genes that cause diseases which are due, in part, to the lack of expression of the gene resulting from the premature stop codon. Such diseases include, but are not limited to, cystic fibrosis, muscular dystrophy, heart disease (*e.g.*, familial hypercholesterolemia), p53-associated cancers (*e.g.*, lung, breast, colon, pancreatic, non-Hodgkin's lymphoma, ovarian, and esophageal cancer), colorectal carcinomas, neurofibromatosis, retinoblastoma, Wilm's tumor, retinitis pigmentosa, collagen disorders (*e.g.*, osteogenesis imperfecta and cirrhosis), Tay Sachs disease, blood disorders (*e.g.*, hemophilia, von Willebrand disease, b-Thalassemia), kidney stones, ataxia-telangiectasia, lysosomal storage diseases, and tuberous sclerosis. Genes involved in the etiology of these diseases are discussed below.

The recognition of translation termination signals is not necessarily limited to a simple trinucleotide stop codon, but is instead recognized by the sequences surrounding the stop codon in addition to the stop codon itself (see, *e.g.*, Manuvakhova *et al.*, 2000, RNA 6(7):1044-1055, which is hereby incorporated by reference in its entirety). Thus, any genes containing particular tetranucleotide sequences at the stop codon, such as, but not limited to, UGAC, UAGU, UAGC, UAGG, UAGA, UGAA, UGAG, UGAU, UAAC, UAAU, UAAG, and UAAA, are candidates of naturally occurring genes with premature stop codons that are useful in the present invention. Human disease genes that contain these particular sequence motifs are sorted by chromosome is presented as an Example in Section 8.

5.5.1. Cystic Fibrosis

Cystic fibrosis is caused by mutations in the cystic fibrosis conductance regulator ("CFTR") gene. Such mutations vary between populations and depend on a multitude of factors such as, but not limited to, ethnic background and geographic location.

Nonsense mutations in the CFTR gene are expected to produce little or not CFTR chloride channels. Several nonsense mutations in the CFTR gene have been identified (see, *e.g.*, Tzetis *et al.*, 2001, Hum Genet. 109(6):592-601. Strandvik *et al.*, 2001, Genet Test. 5(3):235-42; Feldmann *et al.*, 2001, Hum Mutat. 17(4):356; Wilschanski *et al.*, 2000, Am J Respir Crit Care Med. 161(3 Pt 1):860-5; Castaldo *et al.*, 1999, Hum Mutat. 14(3):272; Mittre *et al.*, 1999, Hum Mutat. 14(2):182; Mickle *et al.*, 1998, Hum Mol Genet. 7(4):729-35; Casals *et al.*, 1997, Hum Genet. 101(3):365-70; Mittre *et al.*, 1996, Hum Mutat. 8(4):392-3; Bonizzato *et al.*, 1995, Hum Genet. 1995 Apr;95(4):397-402; Greil *et al.*, 1995, Wien Klin Wochenschr. 107(15):464-9; Zielenski *et al.*, 1995, Hum Mutat. 5(1):43-7; Dork *et al.*, 1994, Hum Genet. 94(5):533-42; Balassopoulou *et al.*, 1994, Hum Mol Genet. 3(10):1887-8; Ghanem *et al.*, 1994, 21(2):434-6; Will *et al.*, J Clin Invest. 1994 Apr;93(4):1852-9; Hull *et al.*, 1994, Genomics. 1994 Jan 15;19(2):362-4; Dork *et al.*, 1994, Hum Genet. 93(1):67-73; Rolfini & Cabrini, 1993, J Clin Invest. 92(6):2683-7; Will *et al.*, 1993, J Med Genet. 30(10):833-7; Bienvenu *et al.*, 1993, J Med Genet. 30(7):621-2; Cheadle *et al.*, 1993, Hum Mol Genet. 2(7):1067-8; Casals *et al.*, 1993, Hum Genet. 91(1):66-70; Reiss *et al.*, 1993, Hum Genet. 91(1):78-9; Chevalier-Porst *et al.*, 1992, Hum Mol Genet. 1(8):647-8; Hamosh *et al.*, 1992, Hum Mol Genet. 1(7):542-4; Gasparini *et al.*, 1992, J Med Genet. 29(8):558-62; Fanen *et al.*, 1992, Genomics. 13(3):770-6; Jones *et al.*, 1992, Hum Mol Genet. 1(1):11-7; Ronchetto *et al.*, 1992, Genomics. 12(2):417-8.; Macek *et al.*, 1992, Hum Mutat. 1(6):501-2; Shoshani *et al.*, 1992, Am J Hum Genet. 50(1):222-8; Schloesser *et al.*, 1991, J Med Genet. 28(12):878-80; Hamosh *et al.*, 1991, J Clin Invest. 88(6):1880-5; Bal *et al.*, 1991, J Med Genet. 28(10):715-7; Dork *et al.*, 1991, Hum Genet. 87(4):441-6; Beaudet *et al.*, 1991, Am J Hum Genet. 48(6):1213; Gasparini *et al.*, 1991, Genomics. 10(1):193-200; Cutting *et al.*, 1990, N Engl J Med. 1990, 323(24):1685-9; and Kerem *et al.*, 1990, Proc Natl Acad Sci U S A. 87(21):8447-51, the disclosures of which are hereby incorporated by reference in their entireties). Any CFTR gene encoding a premature translation codon including, but not limited to, the nonsense mutations described in the references cited above, can be used in the present invention to identify compounds that mediate premature translation termination and/or nonsense-mediated mRNA decay.

5.5.2. Muscular Dystrophy

Muscular dystrophy is a genetic disease characterized by severe, progressive muscle wasting and weakness. Duchenne muscular dystrophy and Becker muscular dystrophy are generally caused by nonsense mutations of the dystrophin gene (see, e.g., Kerr et al., 2001, Hum Genet. 109(4):402-7 and Wagner et al., 2001, Ann Neurol.

49(6):706-11). Nonsense mutations in other genes have also been implicated in other types of muscular dystrophy, such as, but not limited to, collagen genes in Ullrich congenital muscular dystrophy (see, *e.g.*, Demir *et al.*, 2002, Am J Hum Genet. 70(6):1446-58), the emerin gene and lamins genes in Emery-Dreifuss muscular dystrophy (see, *e.g.*, Holt et al., 2001, Biochem Biophys Res Commun. 287(5):1129-33; Becane et al., 2000, Pacing Clin Electrophysiol. 23(11 Pt 1):1661-6; and Bonne *et al.*, 2000, Ann Neurol. 48(2):170-80.), the dysferlin gene in Miyoshi myopathy (see, *e.g.*, Nakagawa et al., 2001, J Neurol Sci. 184(1):15-9), the plectin gene in late onset muscular dystrophy (see, *e.g.*, Bauer et al., 2001, Am J Pathol. 158(2):617-25), the delta-sarcoglycan gene in recessive limb-girdle muscular dystrophy (see, *e.g.*, Duggan *et al.*, 1997, Neurogenetics. 1(1):49-58), the laminina2-chain gene in congenital muscular dystrophy (see, *e.g.*, Mendell et al., 1998, Hum Mutat. 12(2):135), the plectin gene in late-onset muscular dystrophy (see, *e.g.*, Rouan et al., 2000, J Invest Dermatol. 114(2):381-7 and Kunz et al., 2000, J Invest Dermatol. 114(2):376-80), the myophosphorylase gene in McArdle's disease (see, *e.g.*, Bruno *et al.*, 1999, Neuromuscul Disord. 9(1):34-7), and the collagen VI in Bethlem myopathy (see, *e.g.*, Lamande et al., 1998, Hum Mol Genet. 1998 Jun;7(6):981-9).

Several nonsense mutations in the dystrophin gene have been identified (see, *e.g.*, Kerr *et al.*, 2001, Hum Genet. 109(4):402-7; Mendell *et al.*, 2001, Neurology 57(4):645-50; Fajkusova *et al.*, 2001, Neuromuscul Disord. 11(2):133-8; Ginjaar *et al.*, 2000, Eur J Hum Genet. 8(10):793-6; Lu *et al.*, 2000, J Cell Biol. 148(5):985-96; Tuffery-Giraud *et al.*, 1999, Hum Mutat. 14(5):359-68; Fajkusova *et al.*, 1998, J Neurogenet. 12(3):183-9; Tuffery *et al.*, 1998, Hum Genet. 102(3):334-42; Shiga *et al.*, 1997, J Clin Invest. 100(9):2204-10; Winnard *et al.*, 1995, Am J Hum Genet. 56(1):158-66; Prior *et al.*, 1994, Am J Med Genet. 50(1):68-73; Prior *et al.*, 1993, Hum Mol Genet. 2(3):311-3; Prior *et al.*, 1993, Hum Mutat. 2(3):192-5; Nigro et al., 1992, Hum Mol Genet. 1(7):517-20; Worton, 1992, J Inherit Metab Dis. 15(4):539-50; and Bulman *et al.*, 1991, Genomics. 10(2):457-60; the disclosures of which are hereby incorporated by reference in their entireties). Any gene encoding a premature translation codon implicated in muscular dystrophy including, but not limited to, the nonsense mutations described in the references cited above, can be used in the present invention to identify compounds that mediate premature translation termination and/or nonsense-mediated mRNA decay.

5.5.3. Familial Hypercholesterolemia

Hypercholesterolemia, or high blood cholesterol, results from either the overproduction or the underutilization of low density lipoprotein ("LDL").

Hypercholesterolemia is caused by either the genetic disease familial hypercholesterolemia or the consumption of a high cholesterol diet. Nonsense mutations in the LDL receptor gene have been implicated in familial hypercholesterolemia. Several nonsense mutations in the LDL receptor gene have been identified (see, *e.g.*, Lind *et al.*, 2002, Atherosclerosis 163(2):399-407; Salazar *et al.*, 2002, Hum Mutat. 19(4):462-3; Kuhrova *et al.*, 2002, Hum Mutat. 19(1):80; Zakharova *et al.*, 2001, Bioorg Khim. 27(5):393-6; Kuhrova *et al.*, 2001, Hum Mutat. 18(3):253; Genschel *et al.*, 2001, Hum Mutat. 17(4):354; Weiss *et al.*, 2000, J Inherit Metab Dis. 23(8):778-90; Mozas *et al.*, 2000, Hum Mutat. 15(5):483-4; Shin *et al.*, 2000, Clin Genet. 57(3):225-9; Graham *et al.*, 1999, Atherosclerosis 147(2):309-16; Hattori *et al.*, 1999, Hum Mutat. 14(1):87; Cenarro et al., 1998, Hum Mutat. 11(5):413; Rodningen *et al.*, 1999, Hum Mutat. 13(3):186-96; Hirayama *et al.*, 1998, J Hum Genet. 43(4):250-4; Lind *et al.*, 1998, J Intern Med. 244(1):19-25; Thiart *et al.*, 1997, Mol Cell Probes 11(6):457-8; Maruyama *et al.*, 1995, Arterioscler Thromb Vasc Biol. 15(10):1713-8; Koivisto *et al.*, 1995, Am J Hum Genet. 57(4):789-97; Lombardi *et al.*, 1995, J Lipid Res. 36(4):860-7; Leren *et al.*, 1993, Hum Genet. 92(1):6-10; Landsberger et al., 1992, Am J Hum Genet. 50(2):427-33; Loux et al., 1992, Hum Mutat. 1992;1(4):325-32; Motulsky, 1989, Arteriosclerosis. 9(1 Suppl):I3-7; Lehrman *et al.*, 1987, J Biol Chem. 262(1):401-10; and Lehrman *et al.*, 1985, Cell 41(3):735-43; the disclosures of which are hereby incorporated by reference in their entireties). Any LDL receptor gene encoding a premature translation codon including, but not limited to, the nonsense mutations described in the references cited above, can be used in the present invention to identify compounds that mediate premature translation termination and/or nonsense-mediated mRNA decay.

5.5.4. p53-associated Cancers

Mutant forms of the p53 protein, which is thought to act as a negative regulator of cell proliferation, transformation, and tumorigenesis, have been implicated as a common genetic change characteristic of human cancer (see, *e.g.*, Levine *et al.*, 1991, Nature 351:453-456 and Hollstein *et al.*, 1991, Science 253:49-53). p53 mutations have been implicated in cancers such as, but not limited to, lung cancer, breast cancer, colon cancer, pancreatic cancer, non-Hodgkin's lymphoma, ovarian cancer, and esophageal cancer.

Nonsense mutations have been identified in the p53 gene and have been implicated in cancer. Several nonsense mutations in the p53 gene have been identified (see, *e.g.*, Masuda *et al.*, 2000, Tokai J Exp Clin Med. 25(2):69-77; Oh *et al.*, 2000, Mol Cells 10(3):275-80; Li *et al.*, 2000, Lab Invest. 80(4):493-9; Yang *et al.*, 1999, Zhonghua Zhong

Liu Za Zhi 21(2):114-8; Finkelstein *et al.*, 1998, Mol Diagn. 3(1):37-41; Kajiyama *et al.*, 1998, Dis Esophagus. 11(4):279-83; Kawamura *et al.*, 1999, Leuk Res. 23(2):115-26; Radig *et al.*, 1998, Hum Pathol. 29(11):1310-6; Schuyer *et al.*, 1998, Int J Cancer 76(3):299-303; Wang-Gohrke *et al.*, 1998, Oncol Rep. 5(1):65-8; Fulop *et al.*, 1998, J Reprod Med. 43(2):119-27; Ninomiya *et al.*, 1997, J Dermatol Sci. 14(3):173-8; Hsieh *et al.*, 1996, Cancer Lett. 100(1-2):107-13; Rall *et al.*, 1996, Pancreas. 12(1):10-7; Fukutomi *et al.*, 1995, Nippon Rinsho. 53(11):2764-8; Frebourg et al., 1995, Am J Hum Genet. 56(3):608-15; Dove *et al.*, 1995, Cancer Surv. 25:335-55; Adamson *et al.*, 1995, Br J Haematol. 89(1):61-6; Grayson et al., 1994, Am J Pediatr Hematol Oncol. 16(4):341-7; Lepelley *et al.*, 1994, Leukemia. 8(8):1342-9; McIntyre *et al.*, 1994, J Clin Oncol. 12(5):925-30; Horio *et al.*, 1994, Oncogene. 9(4):1231-5; Nakamura *et al.*, 1992, Jpn J Cancer Res. 83(12):1293-8; Davidoff et al., 1992, Oncogene. 7(1):127-33; and Ishioka *et al.*, 1991, Biochem Biophys Res Commun. 177(3):901-6; the disclosures of which are hereby incorporated by reference in their entireties). Any p53 gene encoding a premature translation codon including, but not limited to, the nonsense mutations described in the references cited above, can be used in the present invention to identify compounds that mediate premature translation termination and/or nonsense-mediated mRNA decay.

5.5.5. Colorectal Carcinomas

Molecular genetic abnormalities resulting in colorectal carcinoma involve tumor-suppressor genes that undergo inactivation (such as, but not limited to, apc, mcc, dcc, p53, and possibly genes on chromosomes 8p, 1p, and 22q) and dominant-acting oncogenes (such, but not limited to, ras, src, and myc) (see, *e.g.*, Hamilton, 1992, Cancer 70(5 Suppl):1216-21). Nonsense mutations in the adenomatous polyposis coli ("APC") gene and mismatch repair genes (such as, but not limited to, mlh1 and msh2) have also been described. Nonsense mutations have been implicated in colorectal carcinomas (see, *e.g.*, Viel *et al.*, 1997, Genes Chromosomes Cancer. 18(1):8-18; Akiyama *et al.*, 1996, Cancer 78(12):2478-84; Itoh & Imai , 1996, Hokkaido Igaku Zasshi 71(1):9-14; Kolodner *et al.*, 1994, Genomics. 24(3):516-26; Ohue *et al.*, 1994, Cancer Res. 54(17):4798-804; and Yin *et al.*, 1993, Gastroenterology. 104(6):1633-9; the disclosures of which are hereby incorporated by reference in their entireties). Any gene encoding a premature translation codon implicated in colorectal carcinoma including, but not limited to, the nonsense mutations described in the references cited above, can be used in the present invention to identify compounds that mediate premature translation termination and/or nonsense-mediated mRNA decay.

5.5.6. Neurofibromatosis

Neurofibromatosis is an inherited disorder, which is commonly caused caused by mutations in the NF1 and NF2 tumor suppressor genes. It is characterized by multiple intracranial tumors including schwannomas, meningiomas, and ependymomas. Nonsense mutations in the NF1 and NF2 genes have been described. Nonsense mutations have been implicated in neurofibromatosis (see, *e.g.*, Lamszus *et al.*, 2001, Int J Cancer 91(6):803-8; Sestini *et al.*, 2000, Hum Genet. 107(4):366-71; Fukasawa *et al.*, 2000, Jpn J Cancer Res. 91(12):1241-9; Park *et al.*, 2000, J Hum Genet. 45(2):84-5; Ueki *et al.*, 1999, Cancer Res. 59(23):5995-8; , 1999, Hokkaido Igaku Zasshi. 74(5):377-86; Buske *et al.*, 1999, Am J Med Genet. 86(4):328-30; Harada *et al.*, 1999, Surg Neurol. 51(5):528-35; Krkljus *et al.*, 1998, Hum Mutat. 11(5):411; Klose *et al.*, 1999, Am J Med Genet. 83(1):6-12; Park & Pivnick, 1998, J Med Genet. 35(10):813-20; Bahuau *et al.*, 1998, Am J Med Genet. 75(3):265-72; Bijlsma *et al.*, 1997, J Med Genet. 34(11):934-6; MacCollin *et al.*, 1996, Ann Neurol. 40(3):440-5; Upadhyaya *et al.*, 1996, Am J Med Genet. 67(4):421-3; Robinson *et al.*, 1995, Hum Genet. 96(1):95-8.; Legius *et al.*, 1995, J Med Genet. 32(4):316-9; von Deimling *et al.*, 1995, Brain Pathol. 5(1):11-4; Dublin *et al.*, 1995, Hum Mutat. 5(1):81-5; Legius *et al.*, 1994, Genes Chromosomes Cancer. 10(4):250-5; Purandare *et al.*, 1994, Hum Mol Genet. 3(7):1109-15; Shen & Upadhyaya, 1993, Hum Genet. 92(4):410-2; and Estivill *et al.*, 1991, Hum Genet. 88(2):185-8; the disclosures of which are hereby incorporated by reference in their entireties). Any gene encoding a premature translation codon implicated in neurofibromatosis including, but not limited to, the nonsense mutations described in the references cited above, can be used in the present invention to identify compounds that mediate premature translation termination and/or nonsense-mediated mRNA decay.

5.5.7. Retinoblastoma

The retinoblastoma gene plays important roles in the genesis of human cancers. Several pieces of evidence have shown that the retinoblastoma protein has dual roles in gating cell cycle progression and promoting cellular differentiation (see, *e.g.*, Lee & Lee, 1997, Gan To Kagaku Ryoho 24(11):1368-80 for a review). Nonsense mutations in the RB1 gene have been described. Nonsense mutations have been implicated in retinoblastoma (see, *e.g.*, Klutz et al., 2002, Am J Hum Genet. 71(1):174-9; Alonso et al., 2001, Hum Mutat. 17(5):412-22; Wong et al., 2000, Cancer Res. 60(21):6171-7; Harbour , 1998, Ophthalmology 105(8):1442-7; Fulop et al., 1998, J Reprod Med. 43(2):119-27; Onadim et al., 1997, Br J Cancer 76(11):1405-9; Lohmann et al., 1997, Ophthalmologe

94(4):263-7; Cowell & Cragg, 1996, Eur J Cancer. 32A(10):1749-52; Lohmann et al., 1996, Am J Hum Genet. 58(5):940-9; Shapiro et al., 1995, Cancer Res. 55(24):6200-9; Huang et al., 1993, Cancer Res. 53(8):1889-94; and Cheng & Haas, 1990, Mol Cell Biol. 10(10):5502-9; the disclosures of which are hereby incorporated by reference in their entireties). Any gene encoding a premature translation codon implicated in retinoblastoma including, but not limited to, the nonsense mutations described in the references cited above, can be used in the present invention to identify compounds that mediate premature translation termination and/or nonsense-mediated mRNA decay.

5.5.8. Wilm's Tumor

Wilm's tumor, or nephroblastoma, is an embryonal malignancy of the kidney that affects children. Nonsense mutations in the WT1 gene have been implicated in Wilm's tumor. Several nonsense mutations in the WT1 have been identified (see, *e.g.*, Nakadate *et al.*, 1999, Genes Chromosomes Cancer 25(1):26-32; Diller *et al.*, 1998, J Clin Oncol. 16(11):3634-40; Schumacher *et al.*, 1997, Proc Natl Acad Sci U S A. 94(8):3972-7; Coppes *et al.*, 1993, Proc Natl Acad Sci U S A. 90(4):1416-9; and Little *et al.*, 1992, Proc Natl Acad Sci U S A. 89(11):4791-5; the disclosures of which are hereby incorporated by reference in their entireties). Any WT1 gene encoding a premature translation codon including, but not limited to, the nonsense mutations described in the references cited above, can be used in the present invention to identify compounds that mediate premature translation termination and/or nonsense-mediated mRNA decay.

5.5.9. Retinitis Pigmentosa

Retinitis pigmentosa is a genetic disease in which affected individuals develop progressive degeneration of the rod and cone photoreceptors. Retinitis pigmentosa cannot be explained by a single genetic defect but instead the hereditary aberration responsible for triggering the onset of the disease is localized in different genes and at different sites within these genes (reviewed in, *e.g.*, Kohler *et al.*, 1997, Klin Monatsbl Augenheilkd 211(2):84-93). Nonsense mutations have been implicated in retinitis pigmentosa (see, *e.g.*, Ching *et al.*, 2002, Neurology 58(11):1673-4; Zhang *et al.*, 2002, Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 19(3):194-7; Zhang *et al.*, 2002, Hum Mol Genet. 1;11(9):993-1003; Dietrich *et al.*, 2002, Br J Ophthalmol. 86(3):328-32; Grayson *et al.*, 2002, J Med Genet. 39(1):62-7; Liu *et al.*, 2001, Zhonghua Yi Xue Za Zhi 81(2):71-2; Damji *et al.*, 2001, Can J Ophthalmol. 36(5):252-9; Berson *et al.*, 2001, Invest Ophthalmol Vis Sci. 42(10):2217-24; Chan *et al.*, 2001, Br J Ophthalmol. 85(9):1046-8; Baum *et al.*,

2001, Hum Mutat. 17(5):436; Mashima *et al.*, 2001, Ophthalmic Genet. 22(1):43-7; Zwaenepoel *et al.*, 2001, Hum Mutat. 2001;17(1):34-41; Bork *et al.*, 2001, Am J Hum Genet. 68(1):26-37; Sharon *et al.*, 2000, Invest Ophthalmol Vis Sci. 41(9):2712-21; Dreyer *et al.*, 2000, Eur J Hum Genet. 8(7):500-6; Liu *et al.*, 2000, Hum Mutat. 15(6):584; Wang *et al.*, 1999, Exp Eye Res. 69(4):; Bowne *et al.*, 1999, Hum Mol Genet. 8(11):2121-8; Guillonneau *et al.*, 1999, Hum Mol Genet. 8(8):1541-6; Dryja *et al.*, 1999, Invest Ophthalmol Vis Sci. 40(8):1859-65; Sullivan *et al.*, 1999, Nat Genet. 22(3):255-9; Pierce *et al.*, 1999, Nat Genet. 22(3):248-54; Janecke *et al.*, 1999, Hum Mutat. 13(2):133-40; Cuevas *et al.*, 1998, Mol Cell Probes 12(6):417-20; Schwahn *et al.*, 1998, Nat Genet. 19(4):327-32; Buraczynska *et al.*, 1997, Am J Hum Genet. 61(6):1287-92; Meindl *et al.*, 1996, Nat Genet. 13(1):35-42; Keen *et al.*, 1996, Hum Mutat. 8(4):297-303; Dryja *et al.*, 1995, Proc Natl Acad Sci U S A. 92(22):10177-81; Apfelstedt-Sylla *et al.*, 1995, Br J Ophthalmol. 79(1):28-34; Bayes *et al.*, 1995, Hum Mutat. 5(3):228-34; Shastry, 1994, Am J Med Genet. 52(4):467-74; Gal *et al.*, 1994, Nat Genet. 7(1):64-8; Sargan *et al.*, 1994, Gene Ther. 1 Suppl 1:S89; McLaughlin *et al.*, 1993, Nat Genet. 4(2):130-4; Rosenfeld *et al.*, 1992, Nat Genet. 1(3):209-13; the disclosures of which are hereby incorporated by reference in their entireties). Any gene encoding a premature translation codon implicated in retinitis pigmentosa including, but not limited to, the nonsense mutations described in the references cited above, can be used in the present invention to identify compounds that mediate premature translation termination and/or nonsense-mediated mRNA decay.

5.5.10. Osteogenesis Imperfecta

Osteogenesis imperfecta is a heterogeneous disorder of type I collagen resulting in varying degrees of severity and results from mutations the genes that encode the proalpha chains of type I collagen. Nonsense mutations have been implicated in the genes that encode the proalpha chains of type I collagen ("COLA1" genes) (see, *e.g.*, Slayton *et al.*, 2000, Matrix Biol. 19(1):1-9; Bateman *et al.*, 1999, Hum Mutat. 13(4):311-7; and Willing *et al.*, 1996, Am J Hum Genet. 59(4):799-809; the disclosures of which are hereby incorporated by reference in their entireties). Any COLA1 gene encoding a premature translation codon including, but not limited to, the nonsense mutations described in the references cited above, can be used in the present invention to identify compounds that mediate premature translation termination and/or nonsense-mediated mRNA decay.

5.5.11. Cirrhosis

Cirrhosis generally refers to a chronic liver disease that is marked by replacement of normal tissue with fibrous tissue. The multidrug resistance 3 gene has been implicated in cirrhosis, and nonsense mutations have been identified in this gene (see, *e.g.*, Jacquemin *et al.*, 2001, Gastroenterology. 2001 May;120(6):1448-58; the disclosure of which is hereby incorporated by reference in its entirety). Any gene involved in cirrhosis encoding a premature translation codon including, but not limited to, the nonsense mutations described in the reference cited above, can be used in the present invention to identify compounds that mediate premature translation termination and/or nonsense-mediated mRNA decay.

5.5.12. Tay Sachs Disease

Tay Sachs disease is an autosomal recessive disorder affecting the central nervous system. The disorder results from mutations in the gene encoding the alpha-subunit of beta-hexosaminidase A, a lysosomal enzyme composed of alpha and beta polypeptides. Several nonsense mutations have been implicated in Tay Sachs disease (see, *e.g.*, Rajavel & Neufeld, 2001, Mol Cell Biol. 21(16):5512-9; Myerowitz, 1997, Hum Mutat. 9(3):195-208; Akli *et al.*, 1993, Hum Genet. 90(6):614-20; Mules *et al.*, 1992, Am J Hum Genet. 50(4):834-41; and Akli *et al.*, 1991, Genomics. 11(1):124-34; the disclosures of which are hereby incorporated by reference in their entireties). Any hexosaminidase gene encoding a premature translation codon including, but not limited to, the nonsense mutations described in the references cited above, can be used in the present invention to identify compounds that mediate premature translation termination and/or nonsense-mediated mRNA decay.

5.5.13. Blood Disorders

Hemophilia is caused by a deficiency in blood coagulation factors. Affected individuals are at risk for spontaneous bleeding into organs and treatment usually consists of administration of clotting factors. Hemophilia A is caused by a deficiency of blood coagulation factor VIII and hemophilia B is caused by a deficiency in blood coagulation factor IX. Nonsense mutations in the genes encoding coagulation factors have been implicated in hemophilia (see, *e.g.*, Dansako *et al.*, 2001, Ann Hematol. 80(5):292-4; Moller-Morlang *et al.*, 1999, Hum Mutat. 13(6):504; Kamiya *et al.*, 1998, Rinsho Ketsueki 39(5):402-4; Freson *et al.*, 1998, Hum Mutat. 11(6):470-9; Kamiya *et al.*, 1995, Int J Hematol. 62(3):175-81; Walter *et al.*, 1994, Thromb Haemost. 72(1):74-7; Figueiredo, 1993, Braz J Med Biol Res. 26(9):919-31; Reiner & Thompson, 1992, Hum Genet. 89(1):88-94; Koeberl *et al.*, 1990, Hum Genet. 84(5):387-90; Driscoll *et al.*, 1989, Blood.

74(2):737-42; Chen *et al.*, 1989, Am J Hum Genet. 44(4):567-9; Mikami *et al.*, 1988, Jinrui Idengaku Zasshi. 33(4):409-15; Gitschier *et al.*, 1988, Blood 72(3):1022-8; and Sommer *et al.*, 1987, Mayo Clin Proc. 62(5):387-404; the disclosures of which are hereby incorporated by reference in their entireties). Any gene encoding a premature translation codon implicated in hemophilia including, but not limited to, the nonsense mutations described in the references cited above, can be used in the present invention to identify compounds that mediate premature translation termination and/or nonsense-mediated mRNA decay.

Von Willebrand disease is a single-locus disorder resulting from a deficiency of von Willebrand factor: a multimeric multifunctional protein involved in platelet adhesion and platelet-to-platelet cohesion in high shear stress vessels, and in protecting from proteolysis and directing circulating factor VIII to the site of injury (reviewed in Rodeghiero, 2002, Haemophilia. 8(3):292-300). Nonsense mutations have implicated in von Willehbrand disease (see, *e.g.*, Rodeghiero, 2002, Haemophilia. 8(3):292-300; Enayat *et al.*, 2001, Blood 98(3):674-80; Surdhar *et al.*, 2001, Blood 98(1):248-50; Casana *et al.*, 2000, Br J Haematol. 111(2):552-5; Baronciani *et al.*, 2000, Thromb Haemost. 84(4):536-40; Fellowes *et al.*, 2000, Blood 96(2):773-5; Waseem *et al.*, 1999, Thromb Haemost. 81(6):900-5; Mohlke *et al.*, 1999, Int J Clin Lab Res. 29(1):1-7; Rieger *et al.*, 1998, Thromb Haemost. 80(2):332-7; Kenny *et al.*, 1998, Blood 92(1):175-83; Mazurier *et al.*, 1998, Ann Genet. 41(1):34-43; Hagiwara *et al.*, 1996, Thromb Haemost. 76(2):253-7; Mazurier & Meyer, 1996, Baillieres Clin Haematol. 9(2):229-41; Schneppenheim *et al.*, 1994, Hum Genet. 94(6):640-52; Zhang *et al.*, 1994, Genomics 21(1):188-93; Ginsburg & Sadler, 1993, Thromb Haemost. 69(2):177-84; Eikenboom *et al.*, 1992, Thromb Haemost. 68(4):448-54; Zhang *et al.*, 1992, Am J Hum Genet. 51(4):850-8; Zhang *et al.*, 1992, Hum Mol Genet. 1(1):61-2; and Mancuso *et al.*, 1991, Biochemistry 30(1):253-69; the disclosures of which are hereby incorporated by reference in their entireties). Any gene encoding a premature translation codon implicated in von Willebrand disease including, but not limited to, the nonsense mutations described in the references cited above, can be used in the present invention to identify compounds that mediate premature translation termination and/or nonsense-mediated mRNA decay.

β thalassemia is caused by a deficiency in beta globin polypeptides which in turn causes a deficiency in hemoglobin production. Nonsense mutations have been implicated in b thalassemia (see, *e.g.*, El-Latif *et al.*, 2002, Hemoglobin 26(1):33-40; Sanguansermsri *et al.*, 2001, Hemoglobin 25(1):19-27; Romao 2000, Blood 96(8):2895-901; Perea *et al.*, 1999, Hemoglobin 23(3):231-7; Rhodes *et al.*, 1999, Am J

Med Sci. 317(5):341-5; Fonseca *et al.*, 1998, Hemoglobin 22(3):197-207; Gasperini *et al.*, 1998, Am J Hematol. 1998 Jan;57(1):43-7; Galanello *et al.*, 1997, Br J Haematol. 99(2):433-6; Pistidda *et al.*, 1997, Eur J Haematol. 58(5):320-5; Oner *et al.*, 1997, Br J Haematol. 96(2):229-34; Yasunaga *et al.*, 1995, Intern Med. 34(12):1198-200; Molina *et al.*, 1994, Sangre (Barc) 39(4):253-6; Chang *et al.*, 1994, Int J Hematol. 59(4):267-72; Gilman *et al.*, 1994, Am J Hematol. 45(3):265-7; Chan *et al.*, 1993, Prenat Diagn. 13(10):977-82; George *et al.*, 1993, Med J Malaysia 48(3):325-9; Divoky *et al.*, 1993, Br J Haematol. 83(3):523-4; Fioretti *et al.*, 1993, Hemoglobin 17(1):9-17; Rosatelli *et al.*, 1992, Am J Hum Genet. 50(2):422-6; Moi *et al.*, 1992, Blood 79(2):512-6; Loudianos *et al.*, 1992, Hemoglobin 16(6):503-9; Fukumaki, 1991, Rinsho Ketsueki 32(6):587-91; Cao *et al.*, 1991, Am J Pediatr Hematol Oncol. 13(2):179-88; Galanello *et al.*, 1990, Clin Genet. 38(5):327-31; Liu, 1990, Zhongguo Yi Xue Ke Xue Yuan Xue Bao 12(2):90-5; Aulehla-Scholz *et al.*, 1990, Hum Genet. 84(2):195-7; Cao *et al.*, 1990, Ann N Y Acad Sci. 612:215-25; Sanguansermsri *et al.*, 1990, Hemoglobin 14(2):157-68; Galanello *et al.*, 1989, Blood 74(2):823-7; Rosatelli *et al.*, 1989, Blood 73(2):601-5; Galanello *et al.*, 1989, Prog Clin Biol Res. 316B:113-21; Galanello *et al.*, 1988, Am J Hematol. 29(2):63-6; Chan *et al.*, 1988, Blood 72(4):1420-3; Atweh *et al.*, 1988, J Clin Invest. 82(2):557-61; Masala *et al.*, 1988, Hemoglobin 12(5-6):661-71; Pirastu *et al.*, 1987, Proc Natl Acad Sci U S A 84(9):2882-5; Kazazian *et al.*, 1986, Am J Hum Genet. 38(6):860-7; Cao *et al.*, 1986, Prenat Diagn. 6(3):159-67; Cao *et al.*, 1985, Ann N Y Acad Sci. 1985;445:380-92; Pirastu *et al.*, 1984, Science 223(4639):929-30; Pirastu *et al.*, 1983, N Engl J Med. 309(5):284-7; Trecartin *et al.*, 1981, J Clin Invest. 68(4):1012-7; and Liebhaber *et al.*, 1981, Trans Assoc Am Physicians 94:88-96; the disclosures of which are hereby incorporated by reference in their entireties). Any gene encoding a premature translation codon implicated in b thalassemia including, but not limited to, the nonsense mutations described in the references cited above, can be used in the present invention to identify compounds that mediate premature translation termination and/or nonsense-mediated mRNA decay.

5.5.14. Kidney Stones

Kidney stones (nephrolithiasis), which affect 12% of males and 5% of females in the western world, are familial in 45% of patients and are most commonly associated with hypercalciuria (see, *e.g.*, Lloyd *et al.*, Nature 1996 Feb 1;379(6564):445-9). Mutations of the renal-specific chloride channel gene are associated with hypercalciuric nephrolithiasis (kidney stones). Nonsense mutations have been implicated in kidney stones (see, *e.g.*, Hoopes *et al.*, 1998, Kidney Int. 54(3):698-705; Lloyd *et al.*, 1997, Hum Mol

Genet. 6(8):1233-9; Lloyd *et al.*, 1996, Nature 379(6564):445-9; and Pras *et al.*, 1995, Am J Hum Genet. 56(6):1297-303; the disclosures of which are hereby incorporated by reference in their entireties). Any gene encoding a premature translation codon implicated in kidney stones including, but not limited to, the nonsense mutations described in the references cited above, can be used in the present invention to identify compounds that mediate premature translation termination and/or nonsense-mediated mRNA decay.

5.5.15. Ataxia-Telangiectasia

Ataxia-telangiectasia is characterized by increased sensitivity to ionizing radiation, increased incidence of cancer, and neurodegeneration and is generally caused by mutations in the ataxia-telangiectasia gene (see, *e.g.*, Barlow *et al.*, 1999, Proc Natl Acad Sci USA 96(17):9915-9). Nonsense mutations have been implicated in ataxia-telangiectasia (see, *e.g.*, Camacho *et al.*, 2002, Blood 99(1):238-44; Pitts *et al.*, 2001, Hum Mol Genet. 10(11):1155-62; Laake *et al.*, 2000, Hum Mutat. 16(3):232-46; Li & Swift, 2000, Am J Med Genet. 92(3):170-7; Teraoka *et al.*, 1999, Am J Hum Genet. 64(6):1617-31; and Stoppa-Lyonnet *et al.*, 1998, Blood 91(10):3920-6; the disclosures of which are hereby incorporated by reference in their entireties). Any gene encoding a premature translation codon implicated in ataxia-telangiectasia including, but not limited to, the nonsense mutations described in the references cited above, can be used in the present invention to identify compounds that mediate premature translation termination and/or nonsense-mediated mRNA decay.

5.5.16. Lysosomal Storage Diseases

There are more than 40 individually recognized lysosomal storage disorders. Each disorder results from a deficiency in the activity of a specific enzyme, which impedes the lysosome from carrying out its normal degradative role. These include but are not limited to the diseases listed subsequently. Aspartylglucosaminuria is caused by a deficiency of N-aspartyl-beta-glucosaminidase (Fisher et al., 1990, FEBS Lett. 269:440-444); cholesterol ester storage disease (Wolman disease) is caused by mutations in the LIPA gene (Fujiyama et al., 1996, Hum. Mutat. 8:377-380); mutations in the CTNS gene are associated with cystinosis (Town et al., 1998, Nature Genet. 18:319-324); mutations in a-galactosidase A are associated with Fabry disease (Eng et al.,1993, Pediat. Res. 33:128A; Sakuraba et al., 1990, Am. J. Hum. Genet. 47:784-789; Davies et al., 1993, Hum. Molec. Genet. 2:1051-1053; Miyamura et al., 1996, J. Clin. Invest. 98:1809-1817); fucosidosis is caused by mutations in the FUCA1 gene(Kretz et al., 1989, J. Molec. Neurosci. 1:177-180;

Yang et al., 1992, Biochem. Biophys. Res. Commun. 189:1063-1068; Seo et al., 1993, Hum. Molec. Genet. 2:1205-1208); mucolipidosis type I results from mutations in the NEU1 gene (Bonten et al.,1996, Genes Dev. 10:3156-3169); mucolipidosis type IV results from mutations in theMCOLN1 gene (Bargal et al., 2000, Nature Genet. 26:120-123; Sun et al., 2000,Hum. Molec. Genet. 9:2471-2478); Mucopolysaccharidosis type I (Hurler syndrome) is caused by mutations in the IDUA gene (Scott et al., 1992, Genomics 13:1311-1313;Bach et al., 1993, Am. J. Hum. Genet. 53:330-338); Mucopolysaccharidosis type II (Hunter syndrome) is caused by mutations in the IDS gene (Sukegawa et al., 1992, Biochem. Biophys. Res. Commun. 183:809-813; Bunge et al., 1992 Hum. Molec. Genet. 1:335-339; Flomen et al., 1992, Genomics 13:543-550); mucopolysaccharidosis type 25IIIB (Sanfilippo syndrome type A) is caused by mutations in the SGSH gene(Yogalingam et al., 2001, Hum. Mutat. 18:264-281); mucopolysaccharidosis type IIIB (Sanfilippo syndrome) is caused by mutations in the NAGLU gene (Zhao et al., 1996, Proc. Nat. Acad. Sci. 93:6101-6105; Zhao et al., 1995, Am. J. Hum. Genet. 57:A185); mucopolysaccharidosis type IIID is caused by mutations in the glucosamine-6-sulfatase (G6S) gene (Robertson et al., 1988, Hum. Genet. 79:175-178); mucopolysaccharidosis type IVA (Morquio syndrome) is caused by mutations in the GALNS gene (Tomatsu et al., 1995, Am. J. Hum. Genet. 57:556-563; Tomatsu et al.,1995, Hum. Mutat. 6:195-196); mucopolysaccharidosis type VI (Maroteaux-Lamysyndrome) is caused by mutations in the ARSB gene (Litjens et al., 1992, Hum. Mutat. 1:397-402; Isbrandt et al., 1996, Hum. Mutat. 7:361-363); mucopolysaccharidosis type VII (Sly syndrome) is caused by mutations in the beta-glucuronidase (GUSB) gene(Yamada et al., 1995, Hum. Molec. Genet. 4:651-655); mutations in CLN1 (PPT1) cause infantile neuronal ceroid lipofuscinosis (Das et al., 1998 J. Clin. Invest. 102:361-370; Mitchison et al., 1998, Hum. Molec. Genet. 7:291-297); late infantile type ceroid lipofuscinosis is caused by mutations in the CLN2 gene (Sleat et al., 1997, Science 277:1802-1805); juvenile neuronal ceroid lipofuscinosis (Batten disease) is caused bymutations in the CLN3 gene (Mole et al., 1999, Hum. Mutat. 14: 199-215); late infantileneuronal ceroid lipofuscinosis, Finnish variant, is caused by mutations in the CLN5 gene(Savukoski et al., 1998, Nature Genet. 19:286-288); late-infantile form of neuronal ceroid lipofuscinosis is caused by mutations in the CLN6 gene (Gao et al., 2002, Am. J.Hum. Genet. 70:324-335); Niemann-Pick disease is caused by mutations in the ASM gene (Takahashi et al., 1992, J. Biol. Chem. 267:12552-12558; types A and B) and theNPC1 gene (Millat et al., 2001, Am. J. Hum. Genet. 68:1373-1385; type C); Kanzaki disease is caused by mutations in the NAGA gene (Keulemans et et al., 1996, J. Med.Genet. 33:458-464); Gaucher disease is caused by mutations in the GBA gene (Stone, et al., 1999, Europ.

J. Hum. Genet. 7:505-509); Glycogen storage disease II is the prototypic lysosomal storage disease and is caused by mutations in the GAA gene(Becker et al., 1998, Am. J. Hum. Genet. 62:991-994); Krabbe disease is caused by mutations in the GALC gene (Sakai et al., 1994, Biochem. Biophys. Res. Commun. 198:485-491); Tay-Sachs disease is caused by mutations in the HEXA gene (Akli et al., 1991, Genomics 11:124-134; Mules et al., 1992, Am. J. Hum. Genet. 50: 834-841;Triggs-Raine et al., 1991, Am. J. Hum. Genet. 49:1041-1054; Drucker et al., 1993, Hum. Mutat. 2:415-417; Shore et al., 1992, Hum. Mutat. 1:486-490); mutations in the GM2Agene causes Tay-Sachs variant AB (Schepers et al., 1996, Am. J. Hum. Genet. 59:1048-1056; Chen et al., 1999, Am. J. Hum. Genet. 65:77-87); mutations in the HEXB gene cause Sandhoff disease (Zhang et al., 1994, Hum Mol Genet 3:139-145); alphamannosidosis type II is caused by mutations in the MAN2B1 gene (Gotoda et al., 1998, Am. J. Hum. Genet. 63:1015-1024; Autio et al., 1973, Acta Paediat. Scand. 62:555-565); metachromatic leukodystrophy is caused by mutations in the ARSA gene(Gieselmann et al., 1994, Hum. Mutat. 4:233-242). Any gene containing a premature translation codon implicated in lysosomal storage disease disorders including, but not limited to, the nonsense mutations and genes described in the references cited above, can be used in the present invention to identify compounds that mediate premature translation termination and/or nonsense-mediated mRNA decay.

5.5.17. Tuberous Sclerosis

Tuberous sclerosis complex (TSC) is a dominantly inherited disease characterized by the presence of hamartomata in multiple organ systems. The disease is caused bymutations in TSC1 (van Slegtenhorst *et al.*, 1997 Science 277:805-808; Sato *et al.*, 2002, J. Hum. Genet. 47:20-28) and/or TSC2 (Vrtel *et al.*, 1996, J. Med. Genet. 33:47-51; Wilson *et al.*, 1996, Hum. Molec. Genet. 5:249-256; Au *et al.*, 1998, Am. J.Hum. Genet. 62:286-294; Verhoef *et al.*, 1999, Europ. J. Pediat. 158:284-287;Carsillo *et al.*, 2000, Proc. Nat. Acad. Sci. 97:6085-6090). Any gene containing a premature translation codon implicated in tuberous sclerosis including, but not limited to, the nonsense mutations described in the references cited above, can be used in the present invention to identify compounds that mediate premature translation termination and/or nonsense-mediated mRNA decay.

5.6. Secondary Biological Screens or Assays

5.6.1. *In vitro* Assays

The compounds identified in the assays described supra (for convenience referred to herein as a "lead" compound) can be tested for biological activity using host cells containing or engineered to contain a gene of interest with a premature stop codon or nonsense mutation coupled to a functional readout system. For example, a phenotypic or physiological readout can be used to assess the premature translation termination and/or nonsense-mediated mRNA decay of the RNA product encoded by the gene of interest in the presence and absence of the lead compound.

In one embodiment, a phenotypic or physiological readout can be used to assess the premature translation termination and/or nonsense-mediated mRNA decay of an RNA product of interest in the presence and absence of the lead compound. In accordance with this embodiment, cell-based and cell-free assays described herein, or in International Publication No. WO 01/44516 (which is incorporated herein by reference in its entirety) may be used to assess the premature translation termination and/or nonsense-mediated mRNA decay of the RNA product of interest. Where the gene product of interest is involved in cell growth or viability, the in vivo effect of the lead compound can be assayed by measuring the cell growth or viability of the target cell. Such assays can be carried out with representative cells of cell types involved in a particular disease or disorder (e.g., leukocytes such as T cells, B cells, natural killer cells, macrophages, neutrophils and eosinophils). A lower level of proliferation or survival of the contacted cells indicates that the lead compound is effective to treat a condition in the patient characterized by uncontrolled cell growth. Alternatively, instead of culturing cells from a patient, a lead compound may be screened using cells of a tumor or malignant cell line or an endothelial cell line. Specific examples of cell culture models include, but are not limited to, for lung cancer, primary rat lung tumor cells (see, *e.g.*, Swafford et al., 1997, Mol. Cell. Biol., 17:1366-1374) and large-cell undifferentiated cancer cell lines (see, *e.g.*, Mabry et al., 1991, Cancer Cells, 3:53-58); colorectal cell lines for colon cancer (see, *e.g.*, Park & Gazdar, 1996, J. Cell Biochem. Suppl. 24:131-141); multiple established cell lines for breast cancer (see, e.g., Hambly et al., 1997, Breast Cancer Res. Treat. 43:247-258; Gierthy et al., 1997, Chemosphere 34:1495-1505; and Prasad & Church, 1997, Biochem. Biophys. Res. Commun. 232:14-19); a number of well-characterized cell models for prostate cancer (see, *e.g.*, Webber et al., 1996, Prostate, Part 1, 29:386-394; Part 2, 30:58-64; and Part 3, 30:136-142 and Boulikas, 1997, Anticancer Res. 17:1471-1505); for genitourinary cancers, continuous human bladder cancer cell lines (see, *e.g.*, Ribeiro et al., 1997, Int. J. Radiat. Biol. 72:11-20); organ cultures of transitional cell carcinomas (see, e.g., Booth et al., 1997,

Lab Invest. 76:843-857) and rat progression models (see, *e.g.*, Vet et al., 1997, Biochim. Biophys Acta 1360:39-44); and established cell lines for leukemias and lymphomas (see, *e.g.*, Drexler, 1994, Leuk. Res. 18:919-927 and Tohyama, 1997, Int. J. Hematol. 65:309-317).

Many assays well-known in the art can be used to assess the survival and/or growth of a patient cell or cell line following exposure to a lead compound; for example, cell proliferation can be assayed by measuring bromodeoxyuridine (BrdU) incorporation (see, e.g., Hoshino et al., 1986, Int. J. Cancer 38:369 and Campana et al., 1988, J. Immunol. Meth. 107:79) or (3H)-thymidine incorporation (see, *e.g.*, Chen, 1996, Oncogene 13:1395-403 and Jeoung, 1995, J. Biol. Chem. 270:18367-73), by direct cell count, by detecting changes in transcription, translation or activity of known genes such as proto-oncogenes (e.g., fos, myc) or cell cycle markers (Rb, cdc2, cyclin A, D1, D2, D3, E, etc.). The levels of such protein and mRNA and activity can be determined by any method well known in the art. For example, protein can be quantitated by known immunodiagnostic methods such as western blotting or immunoprecipitation using commercially available antibodies. mRNA can be quantitated using methods that are well known and routine in the art, for example, using northern analysis, RNase protection, the polymerase chain reaction in connection with reverse transcription ("RT-PCR"). Cell viability can be assessed by using trypan-blue staining or other cell death or viability markers known in the art. In a specific embodiment, the level of cellular ATP is measured to determined cell viability. Differentiation can be assessed, for example, visually based on changes in morphology.

The lead compound can also be assessed for its ability to inhibit cell transformation (or progression to malignant phenotype) in vitro. In this embodiment, cells with a transformed cell phenotype are contacted with a lead compound, and examined for change in characteristics associated with a transformed phenotype (a set of in vitro characteristics associated with a tumorigenic ability in vivo), for example, but not limited to, colony formation in soft agar, a more rounded cell morphology, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, release of proteases such as plasminogen activator, increased sugar transport, decreased serum requirement, or expression of fetal antigens, etc. (see, *e.g.*, Luria et al., 1978, General Virology, 3d Ed., John Wiley & Sons, New York, pp. 436-446).

Loss of invasiveness or decreased adhesion can also be assessed to demonstrate the anti-cancer effects of a lead compound. For example, an aspect of the formation of a metastatic cancer is the ability of a precancerous or cancerous cell to detach

from primary site of disease and establish a novel colony of growth at a secondary site. The ability of a cell to invade peripheral sites reflects its potential for a cancerous state. Loss of invasiveness can be measured by a variety of techniques known in the art including, for example, induction of E-cadherin-mediated cell-cell adhesion. Such E-cadherin-mediated adhesion can result in phenotypic reversion and loss of invasiveness (see, *e.g.*, Hordijk et al., 1997, Science 278:1464-66).

Loss of invasiveness can further be examined by inhibition of cell migration. A variety of 2-dimensional and 3-dimensional cellular matrices are commercially available (Calbiochem-Novabiochem Corp. San Diego, CA). Cell migration across or into a matrix can be examined using microscopy, time-lapsed photography or videography, or by any method in the art allowing measurement of cellular migration. In a related embodiment, loss of invasiveness is examined by response to hepatocyte growth factor ("HGF"). HGF-induced cell scattering is correlated with invasiveness of cells such as Madin-Darby canine kidney ("MDCK") cells. This assay identifies a cell population that has lost cell scattering activity in response to HGF (see, *e.g.*, Hordijk et al., 1997, Science 278:1464-66).

Alternatively, loss of invasiveness can be measured by cell migration through a chemotaxis chamber (Neuroprobe/ Precision Biochemicals Inc. Vancouver, BC). In such assay, a chemo-attractant agent is incubated on one side of the chamber (e.g., the bottom chamber) and cells are plated on a filter separating the opposite side (*e.g.*, the top chamber). In order for cells to pass from the top chamber to the bottom chamber, the cells must actively migrate through small pores in the filter. Checkerboard analysis of the number of cells that have migrated can then be correlated with invasiveness (see *e.g.*, Ohnishi, 1993, Biochem. Biophys. Res. Commun.193:518-25).

A lead compound can also be assessed for its ability to alter the expression of a secondary protein (as determined, *e.g.* by western blot analysis) or RNA, whose expression and/or activation is regulated directly or indirectly by the gene product of a gene of interest containing a premature stop codon or a nonsense mutation (as determined, *e.g.*, by RT-PCR or northern blot analysis) in cultured cells *in vitro* using methods which are well known in the art. Further, chemical footprinting analysis can be conducted as described herein (see, *e.g.*, Example 7) or also well-known in the art.

5.6.2. Animal Models

Animal model systems can be used to demonstrate the safety and efficacy of the lead compounds identified in the nonsense suppression assays described above. The

lead compounds identified in the nonsense suppression assay can then be tested for biological activity using animal models for a disease, condition, or syndrome of interest. These include animals engineered to contain the target RNA element coupled to a functional readout system, such as a transgenic mouse.

There are a number of methods that can be used to conduct animal model studies. Briefly, a compound identified in accordance with the methods of the invention is introduced into an animal model so that the effect of the compound on the manifestation of disease can be determined. The prevention or reduction in the severity, duration or onset of a symptom associated with the disease or disorder of the animal model that is associated with, characterized by or caused by premature translation termination and/or nonsense mediated mRNA decay would indicate that the compound adminstered to the animal model had a prophylactic or therapeutic effect. Any method can be used to introduce the compound into the animal model, including, but not limited to, injection, intravenous infusion, oral ingestion, or inhalation. In a preferred embodiment, transgenic hosts are constructed so that the animal genome encodes a gene of interest with a premature translation termination sequence or stop codon. In such an embodiment, the gene, containg a premature translation termination sequence or stop codon, would not encode a full length peptide from a transcribed mRNA. The adminsitration of a compound to the animal model, and the expression of a full length protein, polypeptide or peptide, for example, corresponding to the gene containing a premature stop codon would indicate that the compound modulates premature translation termination. Any method known in the art, or described herein, can be used to determine if the stop codon was modulated by the compound. In another embodiment, an animal is transfected with a reporter construct comprising a regulatory element operably linked to a reporter gene so that the expression the reporter gene is regulated by a regulatory protein or subunit thereof encoded by a nucleic aicd sequence that contains a premature translation termination sequence or stop codon suppression. In such an embodiment, the animal can be cotransfected with a recombinant vector comprising the nucleic acid sequence encoding the regulatory protein with a premature stop codon. In another embodiment, the animal host genome encodes a native gene containing a premature stop codon. In yet another embodiment of the invention, the animal host is a natural mutant, *i.e.*, natively encoding a gene with a premature stop codon. For example, the animal can be a model for cystic fibrosis wherein the animal genome contains a natural mutation that incorporates a premature stop codon or translation termination sequence.

Examples of animal models for cystic fibrosis include, but are not limited to, cftr(-/-) mice (see, *e.g.*, Freedman *et al.*, 2001, Gastroenterology 121(4):950-7), cftr(tm1HGU/tm1HGU) mice (see, *e.g.*, Bernhard *et al.*, 2001, Exp Lung Res 27(4):349-66), CFTR-deficient mice with defective cAMP-mediated Cl(-) conductance (see, *e.g.*, Stotland *et al.*, 2000, Pediatr Pulmonol 30(5):413-24), C57BL/6-Cftr(m1UNC)/Cftr(m1UNC) knockout mice (see, *e.g.*, Stotland *et al.*, 2000, Pediatr Pulmonol 30(5):413-24), an animal model of the human airway, using bronchial xenografts engrafted on rat tracheas and implanted into nude mice (see, *e.g.*, Engelhardt *et al.*, 1992, J. Clin. Invest. 90: 2598-2607), a transgenic mouse model of cystic fibrosis (see, *e.g.*, Clarke *et al.*, 1992, Science 257: 1125-1128; Colledge *et al.*, 1992, Lancet 340: 680 only; Dorin *et al.*, 1992, Nature 359: 211-215; Snouwaert *et al.*, 1992, Science 257: 1083-1088; Manson et al., 1997, EMBO J. 16: 4238-4249).

Examples of animal models for muscular dystrophy include, but are not limited to, mouse, hamster, cat, dog, and *C. elegans*. Examples of mouse models for muscular dystrophy include, but are not limited to, the dy-/- mouse (see, *e.g.*, Connolly *et al.*, 2002, J Neuroimmunol 127(1-2):80-7), a muscular dystrophy with myositis (mdm) mouse mutation (see, *e.g.*, Garvey *et al.*, 2002, Genomics 79(2):146-9), the mdx mouse (see, *e.g.*, Nakamura *et al.*, 2001, Neuromuscul Disord 11(3):251-9), the utrophin-dystrophin knockout (dko) mouse (see, *e.g.*, Nakamura *et al.*, 2001, Neuromuscul Disord 11(3):251-9), the dy/dy mouse (see, *e.g.*, Dubowitz *et al.*, 2000, Neuromuscul Disord 10(4-5):292-8), the mdx(Cv3) mouse model (see, *e.g.*, Pillers *et al.*, 1999, Laryngoscope 109(8):1310-2), and the myotonic ADR-MDX mutant mice (see, *e.g.*, Kramer *et al.*, 1998, Neuromuscul Disord 8(8):542-50). Examples of hamster models for muscular dystrophy include, but are not limited to, sarcoglycan-deficient hamsters (see, *e.g.*, Nakamura *et al.*, 2001, Am J Physiol Cell Physiol 281(2):C690-9) and the BIO 14.6 dystrophic hamster (see, *e.g.*, Schlenker & Burbach, 1991, J Appl Physiol 71(5):1655-62). An example of a feline model for muscular dystrophy includes, but is not limited to, the hypertrophic feline muscular dystrophy model (see, *e.g.*, Gaschen & Burgunder, 2001, Acta Neuropathol (Berl) 101(6):591-600). Canine models for muscular dystrophy include, but are not limited to, golden retriever muscular dystrophy (see, *e.g.*, Fletcher *et al.*, 2001, Neuromuscul Disord 11(3):239-43) and canine X-linked muscular dystrophy (see, *e.g.*, Valentine *et al.*, 1992, Am J Med Genet 42(3):352-6). Examples of *C. elegans* models for muscular dystrophy are described in Chamberlain & Benian, 2000, Curr Biol 10(21):R795-7 and Culette & Sattelle, 2000, Hum Mol Genet 9(6):869-77. Also, a mouse model for Duchenne type muscular

dystrophy has been used to show that treatment with anabolic steroids increases myofiber damage (see, *e.g.*, Krahn *et al.*, 1994, J. Neurol. Sci. 125: 138-146). A feline model for Duchenne type muscular dystrophy has also been described (see, *e.g.*, Winand *et al.*, 1994, 4: 433-445).

Examples of animal models for familial hypercholesterolemia include, but are not limited to, mice lacking functional LDL receptor genes (see, *e.g.*, Aji *et al.*, 1997, Circulation 95(2):430-7), Yoshida rats (see, *e.g.*, Fantappie *et al.*, 1992, Life Sci 50(24):1913-24), the JCR:LA-cp rat (see, *e.g.*, Richardson *et al.*, 1998, Atherosclerosis 138(1):135-46), swine (see, *e.g.*, Hasler-Rapacz *et al.*, 1998, Am J Med Genet 76(5):379-86), the Watanabe heritable hyperlipidaemic rabbit (see, *e.g.*, Tsutsumi *et al.*, 2000, Arzneimittelforschung 50(2):118-21; Harsch *et al.*, 1998, Br J Pharmacol 124(2):227-82; and Tanaka *et al.*, 1995, Atherosclerosis 114(1):73-82); and a family of rhesus monkeys with hypercholesterolemia due to deficiency of the LDL receptor (see, *e.g.*, Scanu *et al.*, 1988, J. Lipid Res. 29: 1671-1681).

An example of an animal model for human cancer in general includes, but is not limited to, spontaneously occurring tumors of companion animals (see, *e.g.*, Vail & MacEwen, 2000, Cancer Invest 18(8):781-92). Examples of animal models for lung cancer include, but are not limited to, lung cancer animal models described by Zhang & Roth (1994, In Vivo 8(5):755-69) and a transgenic mouse model with disrupted p53 function (see, *e.g.*, Morris *et al.*, 1998, J La State Med Soc 150(4):179-85). An example of an animal model for breast cancer includes, but is not limited to, a transgenic mouse that overexpresses cyclin D1 (see, *e.g.*, Hosokawa *et al.*, 2001, Transgenic Res 10(5):471-8). An example of an animal model for colon cancer includes, but is not limited to, a TCRbeta and p53 double knockout mouse (see, *e.g.*, Kado *et al.*, 2001, Cancer Res 61(6):2395-8). Examples of animal models for pancreatic cancer include, but are not limited to, a metastatic model of Panc02 murine pancreatic adenocarcinoma (see, *e.g.*, Wang *et al.*, 2001, Int J Pancreatol 29(1):37-46) and nu-nu mice generated in subcutaneous pancreatic tumours (see, *e.g.*, Ghaneh *et al.*, 2001, Gene Ther 8(3):199-208). Examples of animal models for non-Hodgkin's lymphoma include, but are not limited to, a severe combined immunodeficiency ("SCID") mouse (see, *e.g.*, Bryant *et al.*, 2000, Lab Invest 80(4):553-73) and an IgHmu-HOX11 transgenic mouse (see, *e.g.*, Hough *et al.*, 1998, Proc Natl Acad Sci USA 95(23):13853-8). An example of an animal model for esophageal cancer includes, but is not limited to, a mouse transgenic for the human papillomavirus type 16 E7 oncogene (see, *e.g.*, Herber *et al.*, 1996, J Virol 70(3):1873-81). Examples of animal models for

colorectal carcinomas include, but are not limited to, Apc mouse models (see, *e.g.*, Fodde & Smits, 2001, Trends Mol Med 7(8):369-73 and Kuraguchi *et al.*, 2000, Oncogene 19(50):5755-63). An example of an animal model for neurofibromatosis includes, but is not limited to, mutant NF1 mice (see, *e.g.*, Cichowski *et al.*, 1996, Semin Cancer Biol 7(5):291-8). Examples of animal models for retinoblastoma include, but are not limited to, transgenic mice that expression the simian virus 40 T antigen in the retina (see, *e.g.*, Howes *et al.*, 1994, Invest Ophthalmol Vis Sci 35(2):342-51 and Windle *et al*, 1990, Nature 343(6259):665-9) and inbred rats (see, *e.g.*, Nishida *et al.*, 1981, Curr Eye Res 1(1):53-5 and Kobayashi *et al.*, 1982, Acta Neuropathol (Berl) 57(2-3):203-8). Examples of animal models for Wilm's tumor include, but are not limited to, a WT1 knockout mice (see, *e.g.*, Scharnhorst *et al.*, 1997, Cell Growth Differ 8(2):133-43), a rat subline with a high incidence of neuphroblastoma (see, *e.g.*, Mesfin & Breech, 1996, Lab Anim Sci 46(3):321-6), and a Wistar/Furth rat with Wilms' tumor (see, *e.g.*, Murphy *et al.*, 1987, Anticancer Res 7(4B):717-9).

Examples of animal models for retinitis pigmentosa include, but are not limited to, the Royal College of Surgeons ("RCS") rat (see, *e.g.*, Vollrath *et al.*, 2001, Proc Natl Acad Sci USA 98(22);12584-9 and Hanitzsch *et al.*, 1998, Acta Anat (Basel) 162(2-3):119-26), a rhodopsin knockout mouse (see, *e.g.*, Jaissle *et al.*, 2001, Invest Ophthalmol Vis Sci 42(2):506-13), Wag/Rij rats (see, *e.g.*, Lai *et al.*, 1980, Am J Pathol 98(1):281-4).

Examples of animal models for cirrhosis include, but are not limited to, CCl_4-exposed rats (see, *e.g.*, Kloehn *et al.*, 2001, Horm Metab Res 33(7):394-401) and rodent models instigated by bacterial cell components or colitis (see, *e.g.*, Vierling, 2001, Best Pract Res Clin Gastroenterol 15(4):591-610).

Examples of animal models for hemophilia include, but are not limited to, rodent models for hemophilia A (see, *e.g.*, Reipert *et al.*, 2000, Thromb Haemost 84(5):826-32; Jarvis *et al.*,. 1996, Thromb Haemost 75(2):318-25; and Bi *et al.*, 1995, Nat Genet 10(1):119-21), canine models for hemophilia A (see, *e.g.*, Gallo-Penn *et al.*, 1999, Hum Gene Ther 10(11):1791-802 and Connelly *et al*, 1998, Blood 91(9);3273-81), murine models for hemophilia B (see, *e.g.*, Snyder *et al.*, 1999, Nat Med 5(1):64-70; Wang *et al.*, 1997, Proc Natl Acad Sci USA 94(21):11563-6; and Fang *et al.*, 1996, Gene Ther 3(3):217-22), canine models for hemophilia B (see, *e.g.*, Mount *et al.*, 2002, Blood 99(8):2670-6; Snyder *et al.*, 1999, Nat Med 5(1):64-70; Fang *et al.*, 1996, Gene Ther 3(3):217-22); and Kay *et al.*, 1994, Proc Natl Acad Sci USA 91(6):2353-7), and a rhesus macaque model for hemophilia B (see, *e.g.*, Lozier *et al.*, 1999, Blood 93(6):1875-81).

Examples of animal models for von Willebrand disease include, but are not limited to, an inbred mouse strain RIIIS/J (see, *e.g.*, Nichols *et al.*, 1994, 83(11):3225-31 and Sweeney *et al.*, 1990, 76(11):2258-65), rats injected with botrocetin (see, *e.g.*, Sanders *et al.*, 1988, Lab Invest 59(4):443-52), and porcine models for von Willebrand disease (see, *e.g.*, Nichols *et al.*, 1995, Proc Natl Acad Sci USA 92(7):2455-9; Johnson & Bowie, 1992, J Lab Clin Med 120(4):553-8); and Brinkhous *et al.*, 1991, Mayo Clin Proc 66(7):733-42).

Examples of animal models for b-thalassemia include, but are not limited to, murine models with mutations in globin genes (see, *e.g.*, Lewis *et al.*, 1998, Blood 91(6):2152-6; Raja *et al.*, 1994, Br J Haematol 86(1):156-62; Popp *et al.*, 1985, 445:432-44; and Skow *et al.*, 1983, Cell 34(3):1043-52). Ciavatta and co-workers created a mouse model of beta-zero-thalassemia by targeted deletion of both adult beta-like globin genes, beta(maj) and beta(min), in mouse embryonic stem cells (see, *e.g.*, Ciavatta *et al.*, 1995, Proc Natl Acad Sci U S A. Sep 26;92(20):9259-63).

Examples of animal models for kidney stones include, but are not limited to, genetic hypercalciuric rats (see, *e.g.*, Bushinsky *et al.*, 1999, Kidney Int 55(1):234-43 and Bushinsky *et al.*, 1995, Kidney Int 48(6):1705-13), chemically treated rats (see, *e.g.*, Grases *et al.*, 1998, Scand J Urol Nephrol 32(4):261-5; Burgess *et al.*, 1995, Urol Res 23(4):239-42; Kumar *et al.*, 1991, J Urol 146(5):1384-9; Okada *et al.*, 1985, Hinyokika Kiyo 31(4):565-77; and Bluestone *et al.*, 1975, Lab Invest 33(3):273-9), hyperoxaluric rats (see, *e.g.*, Jones *et al.*, 1991, J Urol 145(4):868-74), pigs with unilateral retrograde flexible nephroscopy (see, *e.g.*, Seifmah *et al.*, 2001, 57(4):832-6), and rabbits with an obstructed upper urinary tract (see, *e.g.*, Itatani *et al.*, 1979, Invest Urol 17(3):234-40).

Examples of animal models for ataxia-telangiectasia include, but are not limited to, murine models of ataxia-telangiectasia (see, *e.g.*, Barlow *et al.*, 1999, Proc Natl Acad Sci USA 96(17):9915-9 and Inoue *et al.*, 1986, Cancer Res 46(8):3979-82). A mouse model was generated for ataxia-telangiectasia using gene targeting to generate mice that did not express the Atm protein (see, *e.g.*, Elson *et al.*, 1996, Proc. Nat. Acad. Sci. 93: 13084-13089).

Examples of animal models for lysosomal storage diseases include, but are not limited to, mouse models for mucopolysaccharidosis type VII (see, *e.g.*, Brooks *et al.*, 2002, Proc Natl Acad Sci U S A. 99(9):6216-21; Monroy *et al.*, 2002, Bone 30(2):352-9; Vogler *et al.*, 2001, Pediatr Dev Pathol. 4(5):421-33; Vogler *et al.*, 2001, Pediatr Res. 49(3):342-8; and Wolfe *et al.*, 2000, Mol Ther. 2(6):552-6), a mouse model for metachromatic leukodystrophy (see, *e.g.*, Matzner *et al.*, 2002, Gene Ther. 9(1):53-63), a

mouse model of Sandhoff disease (see, *e.g.*, Sango *et al.*, 2002, Neuropathol Appl Neurobiol. 28(1):23-34), mouse models for mucopolysaccharidosis type III A (see, *e.g.*, Bhattacharyya *et al.*, 2001, Glycobiology 11(1):99-10 and Bhaumik *et al.*, 1999, Glycobiology 9(12):1389-96.), arylsulfatase A (ASA)-deficient mice (see, *e.g.*, D'Hooge *et al.*, 1999, Brain Res. 847(2):352-6 and D'Hooge *et al*, 1999, Neurosci Lett. 273(2):93-6); mice with an aspartylglucosaminuria mutation (see, *e.g.*, Jalanko et al., 1998, Hum Mol Genet. 7(2):265-72); feline models of mucopolysaccharidosis type VI (see, *e.g.*, Crawley *et al.*, 1998, J Clin Invest. 101(1):109-19 and Norrdin et al., 1995, Bone 17(5):485-9); a feline model of Niemann-Pick disease type C (see, *e.g.*, March *et al.*, 1997, Acta Neuropathol (Berl). 94(2):164-72); acid sphingomyelinase-deficient mice (see, *e.g.*, Otterbach & Stoffel, 1995, Cell 81(7):1053-6), and bovine mannosidosis (see, *e.g.*, Jolly et al., 1975, Birth Defects Orig Artic Ser. 11(6):273-8).

Examples of animal models for tuberous sclerosis ("TSC") include, but are not limited to, a mouse model of TSC1 (see, *e.g.*, Kwiatkowski *et al.*, 2002, Hum Mol Genet. 11(5):525-34), a Tsc1 (TSC1 homologue) knockout mouse (see, *e.g.*, Kobayashi *et al.*, 2001, Proc Natl Acad Sci U S A. 2001 Jul 17;98(15):8762-7), a TSC2 gene mutant(Eker) rat model (see, *e.g.*, Hino 2000, Nippon Rinsho 58(6):1255-61; Mizuguchi *et al.*, 2000, J Neuropathol Exp Neurol. 59(3):188-9; and Hino *et al.*, 1999, Prog Exp Tumor Res. 35:95-108); and Tsc2(+/-) mice (see, *e.g.*, Onda et al., 1999, J Clin Invest. 104(6):687-95).

5.6.3. Toxicity

The toxicity and/or efficacy of a compound identified in accordance with the invention can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). Cells and cell lines that can be used to assess the cytotoxicity of a compound identified in accordance with the invention include, but are not limited to, peripheral blood mononuclear cells (PBMCs), Caco-2 cells, and Huh7 cells. The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. A compound identified in accordance with the invention that exhibits large therapeutic indices is preferred. While a compound identified in accordance with the invention that exhibits toxic side effects may be used, care should be taken to design a delivery system that targets such

agents to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage of a compound identified in accordance with the invention for use in humans. The dosage of such agents lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any agent used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (*i.e.*, the concentration of the compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

5.7. Design of Congeners or Analogs

The compounds which display the desired biological activity can be used as lead compounds for the development or design of congeners or analogs having useful pharmacological activity. For example, once a lead compound is identified, molecular modeling techniques can be used to design variants of the compound that can be more effective. Examples of molecular modeling systems are the CHARM and QUANTA programs (Polygen Corporation, Waltham, MA). CHARM performs the energy minimization and molecular dynamics functions. QUANTA performs the construction, graphic modelling and analysis of molecular structure. QUANTA allows interactive construction, modification, visualization, and analysis of the behavior of molecules with each other.

A number of articles review computer modeling of drugs interactive with specific proteins, such as Rotivinen et al., 1988, Acta Pharmaceutical Fennica 97:159-166; Ripka, 1998, New Scientist 54-57; McKinaly & Rossmann, 1989, Annu. Rev. Pharmacol. Toxiciol. 29:111-122; Perry & Davies, OSAR: Quantitative Structure-Activity Relationships in Drug Design pp. 189-193 (Alan R. Liss, Inc. 1989); Lewis & Dean, 1989, Proc. R. Soc. Lond. 236:125-140 and 141-162; Askew et al., 1989, J. Am. Chem. Soc. 111:1082-1090. Other computer programs that screen and graphically depict chemicals are available from companies such as BioDesign, Inc. (Pasadena, California), Allelix, Inc. (Mississauga, Ontario, Canada), and Hypercube, Inc. (Cambridge, Ontario). Although these

are primarily designed for application to drugs specific to particular proteins, they can be adapted to design of drugs specific to any identified region. The analogs and congeners can be tested for binding to translational machinery using assays well-known in the art or described herein for biologic activity. Alternatively, lead compounds with little or no biologic activity, as ascertained in the screen, can also be used to design analogs and congeners of the compound that have biologic activity.

5.8. Use of Identified Compounds to Treat/Prevent a Disease or Disorder

The present invention provides methods of preventing, treating, managing or ameliorating a disorder associated with premature translation termination and/or nonsense-mediated mRNA decay, or one or more symptoms thereof, said methods comprising administering to a subject in need thereof one or more compounds identified in accordance with the methods of the invention or a pharmaceutically acceptable salt thereof. Examples of diseases associated with, characterized by or caused by associated with premature translation termination and/or nonsense-mediated mRNA decay include, but are not limited to, cystic fibrosis, muscular dystrophy, heart disease, lung cancer, breast cancer, colon cancer, pancreatic cancer, non-Hodgkin's lymphoma, ovarian cancer, esophageal cancer, colorectal carcinomas, neurofibromatosis, retinoblastoma, Wilm's tumor, retinitis pigmentosa, collagen disorders, cirrhosis, Tay-Sachs disease, blood disorders, kidney stones, ataxia-telangiectasia, lysosomal storage diseases, and tuberous sclerosis. See Sections 5.5 and 8 for additional non-limiting examples of diseases and genetic disorders which can be prevented, treated, managed or ameliorated by administering one or more of the compounds identified in accordance with the methods of the invention or a pharmaceutically acceptable salt thereof. Genes that contain one or more nonsense mutations that are potentially involved in causing disease are presented in table form according to chromosome location in Example 8 *infra*.

In a preferred embodiment, it is first determined that the patient is suffering from a disease associated with premature translation termination and/or nonsense-mediated mRNA decay before administering a compound identified in accordance with the invention or a combination therapy described herein. In a preferred embodiment, the DNA of the patient can be sequenced or subject to Southern Blot, polymerase chain reaction (PCR), use of the Short Tandem Repeat (STR), or polymorphic length restriction fragments (RFLP) analysis to determine if a nonsense mutation is present in the DNA of the patient. Alternatively, it can be determined if altered levels of the protein with the nonsense

mutation are expressed in the patient by western blot or other immunoassays. Such methods are well known to one of skill in the art.

In one embodiment, the invention provides a method of preventing, treating, managing or ameliorating a disorder or one or more symptoms thereof, said method comprising administering to a subject in need thereof a dose of a prophylactically or therapeutically effective amount of one or more compounds identified in accordance with the methods of the invention. In another embodiment, a compound identified in accordance with the methods of the invention is not administered to prevent, treat, or ameliorate a disorder or one or more symptoms thereof, if such compound has been used previously to prevent, treat, manage or ameliorate said disorder. In a more specific embodiment of the invention, disorders that can be treated with the compounds of the invention, include, but are not limited to, disorders that are associated with, characterized by or caused by premature translation termination and/or nonsense mediated mRNA decay.

The invention also provides methods of preventing, treating, managing or ameliorating a disorder associated with, characterized by or caused by premature translation termination and/or nonsense mediate mRNA decay, or one or more symptoms thereof, said methods comprising administering to a subject in need thereof one or more of the compounds identified utilizing the screening methods described herein or a pharmaceutically acceptable salt thereof, and one or more other therapies (*e.g.*, prophylactic or therapeutic agents). Preferably, the other therapies are currently being used, have been used or are known to be useful in the prevention, treatment, management or amelioration of said disorder or a symptom thereof. Non-limiting examples of such therapies are in Section 5.8.1 *infra*.

The therapies (*e.g.*, prophylactic or therapeutic agents) or the combination therapies of the invention can be administered sequentially or concurrently. In a specific embodiment, the combination therapies of the invention comprise a compound identified in accordance with the invention and at least one other therapy that has the same mechanism of action as said compound. In another specific embodiment, the combination therapies of the invention comprise a compound identified in accordance with the methods of the invention and at least one other therapy (*e.g.*, prophylactic or therapeutic agent) which has a different mechanism of action than said compound. The combination therapies of the present invention improve the prophylactic or therapeutic effect of a compound of the invention by functioning together with the compound to have an additive or synergistic effect. The

combination therapies of the present invention reduce the side effects associated with the therapies (*e.g.*, prophylactic or therapeutic agents).

The prophylactic or therapeutic agents of the combination therapies can be administered to a subject in the same pharmaceutical composition. Alternatively, the prophylactic or therapeutic agents of the combination therapies can be administered concurrently to a subject in separate pharmaceutical compositions. The prophylactic or therapeutic agents may be administered to a subject by the same or different routes of administration.

In a specific embodiment, a pharmaceutical composition comprising one or more compounds identified in a screening assay described herein is administered to a subject, preferably a human, to prevent, treat, manage or ameliorate a disorder associated with, characterized by or caused by premature translation termination and/or nonsense mediated mRNA decay or one or more symptoms thereof. In accordance with the invention, the pharmaceutical composition may also comprise one or more other prophylactic or therapeutic agents. Preferably, such prophylactic or theapeutic agents are currently being used, have been used or are known to be useful in the prevention, treatment, management or amelioration of a disorder associated with, characterized by, or caused by premature translation termination or nonsense-mediated mRNA decay or one or more symptoms thereof.

A compound identified in accordance with the methods of the invention may be used as a first, second, third, fourth or fifth line of therapy for a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay. The invention provides methods for treating, managing or ameliorating a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay or one or more symptoms thereof in a subject refractory to conventional therapies for such disorder, said methods comprising administering to said subject a dose of a prophylactically or therapeutically effective amount of a compound identified in accordance with the methods of the invention. In particular, a disorder may be determined to be refractory to a therapy when at least some significant portion of the disorder is not resolved in response to the therapy. Such a determination can be made either *in vivo* or *in vitro* by any method known in the art for assaying the effectiveness of a therapy on a subject, using the art-accepted meanings of "refractory" in such a context. In a specific embodiment, a disorder is refractory where the number of symptoms of the disorder has not been significantly reduced, or has increased.

The invention provides methods for treating, managing or ameliorating one or more symptoms of a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay in a subject refractory to existing single agent therapies for such disorder, said methods comprising administering to said subject a dose of a prophylactically or therapeutically effective amount of a compound identified in accordance with the methods of the invention and a dose of a prophylactically or therapeutically effective amount of one or more other therapies (*e.g.*, prophylactic or therapeutic agents). The invention also provides methods for treating or managing a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay by administering a compound identified in accordance with the methods of the invention in combination with any other therapy (*e.g.*, radiation therapy, chemotherapy or surgery) to patients who have proven refractory to other therapies but are no longer on these therapies. The invention also provides methods for the treatment or management of a patient having a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay and said patient is immunosuppressed by reason of having previously undergone other therapies. Further, the invention provides methods for preventing the recurrence of a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay such as, *e.g.*, cancer in patients that have been undergone therapy and have no disease activity by administering a compound identified in accordance with the methods of the invention.

5.8.1. Other Therapies

The present invention provides methods of preventing, treating, managing or ameliorating a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay, or one or more symptoms thereof, said methods comprising administering to a subject in need thereof one or more compounds identified in accordance with the methods of the invention or a pharmaceutically acceptable salt thereof, and one or more other therapies (*e.g.*, prophylactic or therapeutic agents). Any therapy (*e.g.*, chemotherapies, radiation therapies, hormonal therapies, and/or biological therapies/immunotherapies) which is known to be useful, or which has been used or is currently being used for the prevention, treatment, management or amelioration of disorders associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay or one or more symptoms thereof can be used in combination with a compound identified in accordance with the methods of the invention.

Examples of therapeutic or prophylactic agents which can be used in combination with a compound identified in accordance with the invention include, but are not limited to, peptides, polypeptides, fusion proteins, nucleic acid molecules, small molecules, mimetic agents, synthetic drugs, inorganic molecules, and organic molecules.

Proliferative disorders associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay can be prevented, treated, managed or ameliorated by administering to a subject in need thereof one or more of the compounds identified in accordance with the methods of the invention, and one or more other therapies for prevention, treatment, management or amelioration of said disorders or a symptom thereof. Examples of such therapies include, but are not limited to, angiogenesis inhibitors, topoisomerase inhibitors, immunomodulatory agents (such as chemotherapeutic agents) and radiation therapy. Angiogenesis inhibitors (*i.e.*, anti-angiogenic agents) include, but are not limited to, angiostatin (plasminogen fragment); antiangiogenic antithrombin III; angiozyme; ABT-627; Bay 12-9566; Benefin; Bevacizumab; BMS-275291; cartilage-derived inhibitor (CDI); CAI; CD59 complement fragment; CEP-7055; Col 3; combretastatin A-4; endostatin (collagen XVIII fragment); fibronectin fragment; Gro-beta; Halofuginone; Heparinases; Heparin hexasaccharide fragment; HMV833; human chorionic gonadotropin (hCG); IM-862; Interferon alpha/beta/gamma; Interferon inducible protein (IP-10); Interleukin-12; Kringle 5 (plasminogen fragment); Marimastat; Metalloproteinase inhibitors (TIMPs); 2-methoxyestradiol; MMI 270 (CGS 27023A); MoAb IMC-1C11; Neovastat; NM-3; Panzem; PI-88; Placental ribonuclease inhibitor; plasminogen activator inhibitor; platelet factor-4 (PF4); Prinomastat; Prolactin 16kD fragment; Proliferin-related protein (PRP); PTK 787/ZK 222594; retinoids; solimastat; squalamine; SS 3304; SU 5416; SU6668; SU11248; tetrahydrocortisol-S; tetrathiomolybdate; thalidomide; thrombospondin-1 (TSP-1); TNP-470; transforming growth factor-beta; vasculostatin; vasostatin (calreticulin fragment); ZD6126; ZD 6474; farnesyl transferase inhibitors (FTI); and bisphosphonates. In a specific embodiment, anti-angiogenic agents do not include antibodies or fragments thereof that immunospecifically bind to integrin $\alpha_v\beta_3$.

Specific examples of propylactic or therapeutic agents which can be used in accordance with the methods of the invention to prevent, treat, manage or ameliorate a proliferative disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay, or a symptom thereof include, but not limited to: acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin;

altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; flurocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofosine; interleukin II (including recombinant interleukin II, or rIL2), interferon alpha-2a; interferon alpha-2b; interferon alpha-n1 ; interferon alpha-n3; interferon beta-I a; interferon gamma-I b; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine;

vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride. Other anti-cancer drugs include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorlns; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; docetaxel; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine;

ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP

inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; 5-fluorouracil; leucovorin; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; thalidomide; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

Specific examples of propylactic or therapeutic agents which can be used in accordance with the methods of the invention to prevent, treat, manage and/or ameliorate a central nervous system disorders associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay, or a symptom thereof include, but are not limited to: Levodopa, L-DOPA, cocaine, α-methyl-tyrosine, reserpine, tetrabenazine, benzotropine, pargyline, fenodolpam mesylate, cabergoline, pramipexole dihydrochloride, ropinorole, amantadine hydrochloride, selegiline hydrochloride, carbidopa, pergolide mesylate, Sinemet CR, or Symmetrel.

Specific examples of propylactic or therapeutic agents which can be used in accordance with the methods of the invention to prevent, treat, manage and/or ameliorate a metabolic disorders associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay, or a symptom thereof include, but are not limited to: a monoamine oxidase inhibitor (MAO), for example, but not limited to,

iproniazid, clorgyline, phenelzine and isocarboxazid; an acetylcholinesterase inhibitor, for example, but not limited to, physostigmine saliclate, physostigmine sulfate, physostigmine bromide, meostigmine bromide, neostigmine methylsulfate, ambenonim chloride, edrophonium chloride, tacrine, pralidoxime chloride, obidoxime chloride, trimedoxime bromide, diacetyl monoxim, endrophonium, pyridostigmine, and demecarium; an anti-inflammatory agent, including, but not limited to, naproxen sodium, diclofenac sodium, diclofenac potassium, celecoxib, sulindac, oxaprozin, diflunisal, etodolac, meloxicam, ibuprofen, ketoprofen, nabumetone, refecoxib, methotrexate, leflunomide, sulfasalazine, gold salts, RHo-D Immune Globulin, mycophenylate mofetil, cyclosporine, azathioprine, tacrolimus, basiliximab, daclizumab, salicylic acid, acetylsalicylic acid, methyl salicylate, diflunisal, salsalate, olsalazine, sulfasalazine, acetaminophen, indomethacin, sulindac, mefenamic acid, meclofenamate sodium, tolmetin, ketorolac, dichlofenac, flurbinprofen, oxaprozin, piroxicam, meloxicam, ampiroxicam, droxicam, pivoxicam, tenoxicam, phenylbutazone, oxyphenbutazone, antipyrine, aminopyrine, apazone, zileuton, aurothioglucose, gold sodium thiomalate, auranofin, methotrexate, colchicine, allopurinol, probenecid, sulfinpyrazone and benzbromarone or betamethasone and other glucocorticoids; an antiemetic agent, for example, but not limited to, metoclopromide, domperidone, prochlorperazine, promethazine, chlorpromazine, trimethobenzamide, ondansetron, granisetron, hydroxyzine, acetylleucine monoethanolamine, alizapride, azasetron, benzquinamide, bietanautine, bromopride, buclizine, clebopride, cyclizine, dimenhydrinate, diphenidol, dolasetron, meclizine, methallatal, metopimazine, nabilone, oxyperndyl, pipamazine, scopolamine, sulpiride, tetrahydrocannabinol, thiethylperazine, thioproperazine, tropisetron, and mixtures thereof.

5.9. Compounds and Methods of Administering Compounds

Biologically active compounds identified using the methods of the invention or a pharmaceutically acceptable salt thereof can be administered to a patient, preferably a mammal, more preferably a human, suffering from a disorder associated with, characterized by or caused by premature translation termination and/or nonsense mediated mRNA decay. In a specific embodiment, a compound or a pharmaceutically acceptable salt thereof is administered to a patient, preferably a mammal, more preferably a human, as a preventative measure against a disorder associated with, characterized by or caused by premature translation termination and/or nonsense-mediated mRNA decay.

When administered to a patient, the compound or a pharmaceutically acceptable salt thereof is preferably administered as component of a composition that

optionally comprises a pharmaceutically acceptable vehicle. The composition can be administered orally, or by any other convenient route, for example, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal, and intestinal mucosa, *etc.*) and may be administered together with another biologically active agent. Administration can be systemic or local. Various delivery systems are known, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, capsules, *etc.*, and can be used to administer the compound and pharmaceutically acceptable salts thereof.

Methods of administration include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, oral, sublingual, intranasal, intracerebral, intravaginal, transdermal, rectally, by inhalation, or topically, particularly to the ears, nose, eyes, or skin. The mode of administration is left to the discretion of the practitioner. In most instances, administration will result in the release of the compound or a pharmaceutically acceptable salt thereof into the bloodstream.

In specific embodiments, it may be desirable to administer the compound or a pharmaceutically acceptable salt thereof locally. This may be achieved, for example, and not by way of limitation, by local infusion during surgery, topical application, *e.g.*, in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers.

In certain embodiments, it may be desirable to introduce the compound or a pharmaceutically acceptable salt thereof into the central nervous system by any suitable route, including intraventricular, intrathecal and epidural injection. Intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir.

Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent, or via perfusion in a fluorocarbon or synthetic pulmonary surfactant. In certain embodiments, the compound and pharmaceutically acceptable salts thereof can be formulated as a suppository, with traditional binders and vehicles such as triglycerides.

In another embodiment, the compound and pharmaceutically acceptable salts thereof can be delivered in a vesicle, in particular a liposome (see Langer, 1990, Science 249:1527-1533; Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer,

Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*).

In yet another embodiment, the compound and pharmaceutically acceptable salts thereof can be delivered in a controlled release system (see, *e.g.*, Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)). Other controlled-release systems discussed in the review by Langer, 1990, Science 249:1527-1533 may be used. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201; Buchwald et al., 1980, Surgery 88:507; Saudek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, J. Macromol. Sci. Rev. Macromol. Chem. 23:61; see also Levy et al., 1985, Science 228:190; During et al., 1989, Ann. Neurol. 25:351; Howard et al., 1989, J. Neurosurg. 71:105). In yet another embodiment, a controlled-release system can be placed in proximity of a target RNA of the compound or a pharmaceutically acceptable salt thereof, thus requiring only a fraction of the systemic dose.

Compositions comprising the compound or a pharmaceutically acceptable salt thereof ("compound compositions") can additionally comprise a suitable amount of a pharmaceutically acceptable vehicle so as to provide the form for proper administration to the patient.

In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, mammals, and more particularly in humans. The term "vehicle" refers to a diluent, adjuvant, excipient, or carrier with which a compound of the invention is administered. Such pharmaceutical vehicles can be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The pharmaceutical vehicles can be saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents may be used. When administered to a patient, the pharmaceutically acceptable vehicles are preferably sterile. Water is a preferred vehicle when the compound of the invention is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid vehicles,

particularly for injectable solutions. Suitable pharmaceutical vehicles also include excipients such as starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. Compound compositions, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

Compound compositions can take the form of solutions, suspensions, emulsion, tablets, pills, pellets, capsules, capsules containing liquids, powders, sustained-release formulations, suppositories, emulsions, aerosols, sprays, suspensions, or any other form suitable for use. In one embodiment, the pharmaceutically acceptable vehicle is a capsule (see *e.g.*, U.S. Patent No. 5,698,155). Other examples of suitable pharmaceutical vehicles are described in Remington's Pharmaceutical Sciences, Alfonso R. Gennaro, ed., Mack Publishing Co. Easton, PA, 19th ed., 1995, pp. 1447 to 1676, incorporated herein by reference.

In a preferred embodiment, the compound or a pharmaceutically acceptable salt thereof is formulated in accordance with routine procedures as a pharmaceutical composition adapted for oral administration to human beings. Compositions for oral delivery may be in the form of tablets, lozenges, aqueous or oily suspensions, granules, powders, emulsions, capsules, syrups, or elixirs, for example. Orally administered compositions may contain one or more agents, for example, sweetening agents such as fructose, aspartame or saccharin; flavoring agents such as peppermint, oil of wintergreen, or cherry; coloring agents; and preserving agents, to provide a pharmaceutically palatable preparation. Moreover, where in tablet or pill form, the compositions can be coated to delay disintegration and absorption in the gastrointestinal tract thereby providing a sustained action over an extended period of time. Selectively permeable membranes surrounding an osmotically active driving compound are also suitable for orally administered compositions. In these later platforms, fluid from the environment surrounding the capsule is imbibed by the driving compound, which swells to displace the agent or agent composition through an aperture. These delivery platforms can provide an essentially zero order delivery profile as opposed to the spiked profiles of immediate release formulations. A time delay material such as glycerol monostearate or glycerol stearate may also be used. Oral compositions can include standard vehicles such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. Such vehicles are preferably of pharmaceutical grade. Typically, compositions for intravenous administration comprise

sterile isotonic aqueous buffer. Where necessary, the compositions may also include a solubilizing agent.

In another embodiment, the compound or a pharmaceutically acceptable salt thereof can be formulated for intravenous administration. Compositions for intravenous administration may optionally include a local anesthetic such as lignocaine to lessen pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water-free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the compound or a pharmaceutically acceptable salt thereof is to be administered by infusion, it can be dispensed, for example, with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the compound or a pharmaceutically acceptable salt thereof is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The amount of a compound or a pharmaceutically acceptable salt thereof that will be effective in the treatment of a particular disease will depend on the nature of the disease, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed will also depend on the route of administration, and the seriousness of the disease, and should be decided according to the judgment of the practitioner and each patient's circumstances. However, suitable dosage ranges for oral administration are generally about 0.001 milligram to about 500 milligrams of a compound or a pharmaceutically acceptable salt thereof per kilogram body weight per day. In specific preferred embodiments of the invention, the oral dose is about 0.01 milligram to about 100 milligrams per kilogram body weight per day, more preferably about 0.1 milligram to about 75 milligrams per kilogram body weight per day, more preferably about 0.5 milligram to 5 milligrams per kilogram body weight per day. The dosage amounts described herein refer to total amounts administered; that is, if more than one compound is administered, or if a compound is administered with a therapeutic agent, then the preferred dosages correspond to the total amount administered. Oral compositions preferably contain about 10% to about 95% active ingredient by weight.

Suitable dosage ranges for intravenous (i.v.) administration are about 0.01 milligram to about 100 milligrams per kilogram body weight per day, about 0.1 milligram to about 35 milligrams per kilogram body weight per day, and about 1 milligram to about

10 milligrams per kilogram body weight per day. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight per day to about 1 mg/kg body weight per day. Suppositories generally contain about 0.01 milligram to about 50 milligrams of a compound of the invention per kilogram body weight per day and comprise active ingredient in the range of about 0.5% to about 10% by weight.

Recommended dosages for intradermal, intramuscular, intraperitoneal, subcutaneous, epidural, sublingual, intracerebral, intravaginal, transdermal administration or administration by inhalation are in the range of about 0.001 milligram to about 200 milligrams per kilogram of body weight per day. Suitable doses for topical administration are in the range of about 0.001 milligram to about 1 milligram, depending on the area of administration. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems. Such animal models and systems are well known in the art.

The compound and pharmaceutically acceptable salts thereof are preferably assayed *in vitro* and *in vivo*, for the desired therapeutic or prophylactic activity, prior to use in humans. For example, *in vitro* assays can be used to determine whether it is preferable to administer the compound, a pharmaceutically acceptable salt thereof, and/or another therapeutic agent. Animal model systems can be used to demonstrate safety and efficacy.

6. EXAMPLE: PREPARATION OF EXTRACTS FROM HELACELLS FOR INVITRO TRANSLATION REACTIONS

This Example describes a method of preparing a cell extract to perform *in vitro* translation reactions to monitor nonsense suppression or to produce proteins *in vitro*. This method is different from other methods used to prepare translation extracts for several reasons. First, the centrifugation step is performed at low speed (12000Xg) compared to most other protocols that use a 100,000Xg spin; and second, the cells are incubated on ice for several hours to weeks, which increases the activity of the extract significantly.

6.1. Preparation of Translation Extract from Hela Cells

HeLa S3 cells were grown to a density of 10^6 cells/ml in DMEM; 5%CO_2, 10% FBS, 1X P/S in a spinner flask. Cells were harvested by spinning at 1000Xg. Cells were washed twice with phosphate buffered saline. The cell pellet sat on ice for 12 to 24 hours before proceeding. By letting the cells sit on ice, the activity of the extract is increased two-fold. The length of time on ice can range from 0 hours to 1 week. The cells were resuspended in 1.5 volumes (packed cell volume) of hypotonic buffer (10 mM HEPES

(KOH) pH 7.4; 15 mM KCl; 1.5 mM $Mg(OAc)_2$; 0.5 mM Pefabloc (Roche); 2 mM DTT). Cells were allowed to swell for 5 minutes on ice and dounce homogenized with 10 to 100 strokes using a tight-fitting pestle. The cells were spun for 10 minutes at 12000Xg at 4°C in a Sorvall SS-34 rotor. The supernatant was carefully collected with a Pasteur pipet without disturbing the lipid layer and transferred into Eppendorf tubes (50-200 mL aliquots) and immediately frozen in liquid nitrogen. Figure 1 shows the amount of wild-type luciferase produced in an in vitro translation reaction when the amount of wild-type luciferase RNA and the amount of HeLa cell extract are varied. Figure 2 shows the amount of wild-type luciferase produced in an *in vitro* translation reaction when the amount of luciferase RNA containing the nonsense mutation RGA and the amount of the cell are varied.

6.2 Incubating Cells on Ice Improves Translation Activity of Extract

As shown in Figure 3, incubating cells on ice prior to preparation of the translation extract improves the translation activity up to 20 fold. Further, in the presence of the aminoglycoside gentamicin, nonsense suppression activity (as measured by the amount of luciferase activity produced from a luciferase RNA containing a UGA premature termination codon) is increased 2 to 3 fold above untreated extracts (see Figure 4). These results demonstrate that extracts prepared by this method actively translate wild type RNA as well as mediate nonsense suppression.

7. EXAMPLE: IDENTIFICATION AND CHARACTERIZATION OF COMPOUND THAT PROMOTE NONSENSE SUPPRESSION AND/OR MODULATE TRANSLATION TERMINATION

7.1. Development of Assays for High Throughput Screens

Two assays were developed for use in high throughput screens to identify small molecules that promote nonsense suppression. Each assay utilized luciferase because it is a functional reporter gene assay (light is only produced if the protein is functional) and it is extremely sensitive (Light intensity is proportional to luciferase concentration in the nM range). The first assay was a cell-based luciferase reporter assay and the second was a biochemical assay consisting of rabbit reticulocyte lysate and a nonsense-containing luciferase reporter mRNA. In the cell-based assay, a luciferase reporter construct containing a UGA premature termination codon was stably transfected in 293T Human Embryonic Kidney cells. In the biochemical assay, mRNA containing a UGA premature termination codon was used as a reporter in an in vitro translation reaction using rabbit

reticulocyte lysate supplemented with tRNA, hemin, creatine kinase, amino acids, KOAc, $Mg(OAc)_2$, and creatine phosphate. Translation of the mRNA was initiated within a virus derived leader sequence, which significantly reduced the cost of the assay because capped RNA was not required. Synthetic mRNA was prepared *in vitro* using the T7 promoter and the MegaScript in vitro transcription kit (Ambion). In both of the biochemical and cell-based assays, addition of gentamicin, a small molecule known to allow readthrough of premature termination codons, resulted in increased luciferase activity and was, therefore, used as an internal standard.

7.2. Screening of a Chemical Library Using the Nonsense Suppression Assays

The assays described above in Section 7.1 were used in two high throughput screens. Approximately eight hundred thousand compounds were screened in the cell-based and biochemical assays. From these initial screens two hundred hits were retested with both luciferase assays and seven compounds were subsequently selected for further investigation. These compounds fall into four classes of scaffolds. One class of compound is a nucleoside analog; the second class is a quinazoline compound; the third class is an oxadiazole compound similar to diarylfuran antibiotics; and the final class is a unique scaffold harboring one or more phenyl, amide, or similar functional groups. Interestingly, none of the compounds are similar in structure to gentamicin. Compound A (molecular formula $C_{19}H_{21}NO_4$), a member of the fourth class and Compound B (molecular formula $C_{19}H_{18}N_2O_4$), a compound synthesized independently of the screen, because of its potential RNA binding properties were the focus of subsequent attention.

7.3. Compound A and Compound B Increase In Vitro Nonsense Suppression at UGA Codons

Based on the results of the high throughput screen, Compound A was characterized further with the in vitro luciferase nonsense suppression assay. To ensure that the observed nonsense suppression activity of the selected compounds was not limited to the rabbit reticulocyte assay system, HeLa cell extract was prepared and optimized (Lie & Macdonald, 1999, Development 126(22):4989-4996 and Lie & Macdonald, 2000, Biochem. Biophys. Res. Commun. 270(2):473-481). Figure 5 shows that Compound A and Compound B exhibit greater nonsense suppression activity of the UGA codon than gentamicin in the HeLa cell translation extracts.

7.4. Characterization of Compounds That Increase Nonsense Suppression and Product Function Protein

Compound A and Compound B increase the level of nonsense suppression in the biochemical assay three to four fold over untreated extracts. To determine whether these compounds also function *in vivo*, a stable cell line harboring the UGA nonsense-containing luciferase gene was treated with each compound. Cells were grown in standard medium supplemented with 1% penicillin-streptomycin (P/S) and 10% fetal bovine serum (FBS) to 70% confluency and split 1:1 the day before treatment. On the following day, cells were trypsinized and 40,000 cells were added to each well of a 96-well tissue culture dish. Serial dilutions of each compound were prepared to generate a six-point dose response curve spanning 2 logs (30μM to 0.3 μM). The final concentration of the DMSO solvent remained constant at 1% in each well. Cells treated with 1% DMSO served as the background standard, and cells treated with gentamicin served as a positive control. As shown in Figure 6, these two compounds are more potent and efficacious than gentamicin at these concentrations.

Cells were transiently transfected with plasmids harboring the UGA, UAA or UAG nonsense alleles of luciferase in each codon context (UGAA, UGAC, UGAG, UGAU, UAGA, UAGC, UAGG, UAGU, UAAA, UAAC, UAAG, and UAAU) then the cells were treated overnight with Compound A, and gentamicin. The following day, the level of suppression was determined by measuring the amount of luminescence produced. The fold suppression above control cells treated with solvent was calculated and is numerically reported. The results are presented in Table 2 and Figure 6B.

Table 2

Context	Gentamicin 3mg/ml	Compound A 10uM
UAAA	0.71	0.17
UAAC	2.32	0.67
UAAG	0.01	0.02
UAAU	0.92	0.33
UAGA	1.31	0.64
UAGC	2.16	3.05
UAGG	0.64	0.51
UAGU	0.54	0.31
UGAA	0.76	0.4
UGAC	1.91	2.96
UGAG	0.45	0.23
UGAU	6.74	1.67

7.5. Compound A Alters the Accessibility of the Chemical Modifying Agents to Specific Nucleotides in the 28S rRNA

Previous studies have demonstrated that gentamicin and other members of the aminoglycoside family that decrease the fidelity of translation bind to the A site of the 16S rRNA. By chemical footprinting, UV cross-linking and NMR, gentamicin has been shown to bind at the A site (comprised of nucleotides 1400-1410 and 1490-1500, *E. coli* numbering) of the rRNA at nucleotides 1406, 1407, 1494, and 1496 (Moazed & Noller, 1987, Nature 327(6121):389-394; Woodcock *et al.*, 1991, EMBO J. 10(10):3099-3103; and Schroeder *et al.*, 2000). These observations prompted us to determine whether similar experiments could provide information on the mechanism of action of Compound A. To do this, ribosomes prepared from HeLa cells were incubated with the small molecules (at a concentration of 100 μM), followed by treatment with chemical modifying agents (dimethyl sulfate [DMS] and kethoxal [KE]). Following chemical modification, rRNA was phenol-chloroform extracted, ethanol precipitated, analyzed in primer extension reactions using end-labeled oligonucleotides hybridizing to different regions of the three rRNAs and resolved on 6% polyacrylamide gels. The probes used for primer extension cover the entire 18S (7 oligonucleotide primers), 28S (24 oligonucleotide primers), and 5S (one primer) rRNAs. Controls in these experiments include DMSO (a control for changes in rRNA accessibility induced by DMSO), paromomycin (a marker for 18S rRNA binding), and anisomycin (a marker for 28S rRNA binding).

The results of these foot-printing experiments indicated that Compound A alters the accessibility of the chemical modifying agents to specific nucleotides in the 28S rRNA. More specifically, the regions protected by Compound A include: (1) a conserved region in the vicinity of the peptidyl transferase center (domain V) implicated in peptide bond formation (see Figure 7A) and (2) a conserved region in domain II that may interact with the peptidyl transferase center based on binding of vernamycinin B to both these areas (Vannuffel *et al.*, 1994, Nucleic Acids Res. 22(21):4449-4453; see Figure 7B).

7.6. Compound A Causes Readthrough of Premature Termination Codons in Cell-based Disease Models

To address the effects of the nonsense-suppressing compounds on mRNAs altered in specific inherited diseases, a bronchial epithelial cell line harboring a nonsense codon at amino acid 1282 (W1282X) was treated with Compound A (20μM) and CFTR function was monitored as a cAMP-activated chloride channel using the SPQ assay (Yang *et al.*, 1993, Hum Mol Genet. 2(8):1253-1261 and Howard *et al.*, 1996, Nat Med. 2(4):467-469). These experiments showed that cAMP treatment of these cells resulted in an increase in SPQ fluorescence, consistent with stimulation of CFTR-mediated halide efflux

(Figure 8). No increase in fluorescence was observed when cells were not treated with compound or if the cells were not stimulated with cAMP. These results indicate that the full-length CFTR expressed from this nonsense-containing allele following compound treatment also functions as a cAMP-stimulated anion channel, thus demonstrating that cystic fibrosis cell lines increase chloride channel activity when treated with Compound A.

7.7. Primary Cells from the mdx Nonsense-containing Mouse Express Full-length Dystrophin Protein When Treated with Compound A

The mutation in the mdx mouse that premature termination of the 427 kDa dystrophin polypeptide has been shown to be a C to T transition at position 3185 in exon 23 (Sicinski *et al.*, 1989, Science. 244(4912):1578-1580). Mouse primary skeletal muscle cultures derived from 1-day old mdx mice were prepared as described previously (Barton-Davis *et al.*, 1999, J Clin Invest. 104(4):375-381). Cells were cultured for 10 days in the presence of Compound A (20 μM). Culture medium was replaced every four days and the presence of dystrophin in myoblast cultures was detected by immunostaining as described previously (Barton-Davis *et al.*, 1999, J Clin Invest. 104(4):375-381). A primary monoclonal antibody to the C-terminus of the dystrophin protein (F19A12) was used undiluted and rhodamine conjugated anti-mouse IgG was used as the secondary antibody. The F19A12 antibody will detect the full-length protein produced by suppression of the nonsense codon. Staining was viewed using a Leica DMR micropscope, digital camera, and associated imaging software at the University of Pennsylvania. As shown in Figure 9, full-length dystrophin protein is produced and localized to the muscle myotubes in cultures treated with 20 μM Compound A and gentamicin (200 μM). In addition, cells from untreated cultures exhibited minimal staining. These results indicate that full-length dystrophin protein is produced as a consequence of nonsense suppression.

7.8. Compound A and Compound B Cause Readthrough of Premature Termination Codons in the mdx Mouse

Since the results of the mdx cell culture experiments demonstrated production of full-length dystrophin in cells treated with Compound A, it was asked whether suppression of the nonsense codon in the mdx mouse could be observed. As previously described (Barton-Davis *et al.*, 1999, J Clin Invest. 104(4):375-381), compound was delivered by Alzet osmotic pumps implanted under the skin of anesthetized mice. Two doses of Compound A were administered. Gentamicin served as a positive control and pumps filled with solvent only served as the negative control. Pumps were loaded with appropriate compound such that the calculated doses to which tissue was exposed were 10

μM and 20 μM. The gentamicin concentration was calculated to achieve tissue exposure of approximately 200 μM. In the initial experiment, mice were treated for 14 days, after which animals were anesthetized with ketamine and exsanguinated. The tibialis anterior (TA) muscle of the experimental animals was then excised, frozen, and used for immunofluorescence analysis of dystrophin incorporation into striated muscle. The presence of dystrophin in TA muscles was detected by immunostaining, as described previously (Barton-Davis *et al.*, 1999, J Clin Invest. 104(4):375-381; see mdx primary cells in Section 7.8 *supra*). As shown in Figure 10, these experiments demonstrated that mice treated with both concentrations of compound elicited production of full-length dystrophin. Importantly, a significant portion of the full-length dystrophin protein was properly localized to the membrane. These important results demonstrate that Compound A can function in an animal model.

8. HUMAN DISEASE GENES SORTED BY CHROMOSOME

Table 3: Genes, Locations and Genetic Disorders on Chromosome 1

Gene	GDB Accession ID	OMIM Link
ABCA4	GDB:370748	MACULAR DEGENERATION, SENILE STARGARDT DISEASE 1; STGD1 ATP BINDING CASSETTE TRANSPORTER; ABCR RETINITIS PIGMENTOSA-19; RP19
ABCD3	GDB:131485	PEROXISOMAL MEMBRANE PROTEIN 1; PXMP1
ACADM	GDB:118958	ACYL-CoA DEHYDROGENASE, MEDIUM-CHAIN; ACADM
AGL	GDB:132644	GLYCOGEN STORAGE DISEASE III
AGT	GDB:118750	ANGIOTENSIN I; AGT
ALDH4A1	GDB:9958827	HYPERPROLINEMIA, TYPE II

ALPL	GDB:118730	PHOSPHATASE, LIVER ALKALINE; ALPL HYPOPHOSPHATASIA, INFANTILE
AMPD1	GDB:119677	ADENOSINE MONOPHOSPHATE DEAMINASE-1; AMPD1
APOA2	GDB:119685	APOLIPOPROTEIN A-II; APOA2
AVSD1	GDB:265302	ATRIOVENTRICULAR SEPTAL DEFECT; AVSD
BRCD2	GDB:9955322	BREAST CANCER, DUCTAL, 2; BRCD2
C1QA	GDB:119042	COMPLEMENT COMPONENT 1, q SUBCOMPONENT, ALPHA POLYPEPTIDE; C1QA
C1QB	GDB:119043	COMPLEMENT COMPONENT 1, q SUBCOMPONENT, BETA POLYPEPTIDE; C1QB
C1QG	GDB:128132	COMPLEMENT COMPONENT 1, q SUBCOMPONENT, GAMMA POLYPEPTIDE; C1QG
C8A	GDB:119735	COMPLEMENT COMPONENT-8, DEFICIENCY OF
C8B	GDB:119736	COMPLEMENT COMPONENT-8, DEFICIENCY OF, TYPE II
CACNA1S	GDB:126431	CALCIUM CHANNEL, VOLTAGE-DEPENDENT, L TYPE, ALPHA 1S SUBUNIT; CACNA1S PERIODIC PARALYSIS I MALIGNANT HYPERTHERMIA SUSCEPTIBILITY-5; MHS5

CCV	GDB:1336655	CATARACT, CONGENITAL, VOLKMANN TYPE; CCV
CD3Z	GDB:119766	CD3Z ANTIGEN, ZETA POLYPEPTIDE; CD3Z
CDC2L1	GDB:127827	PROTEIN KINASE p58; PK58
CHML	GDB:135222	CHOROIDEREMIA-LIKE; CHML
CHS1	GDB:4568202	CHEDIAK-HIGASHI SYNDROME; CHS1
CIAS1	GDB:9957338	COLD HYPERSENSITIVITY URTICARIA, DEAFNESS, AND AMYLOIDOSIS
CLCNKB	GDB:698472	CHLORIDE CHANNEL, KIDNEY, B; CLCNKB
CMD1A	GDB:434478	CARDIOMYOPATHY, DILATED 1A; CMD1A
CMH2	GDB:137324	CARDIOMYOPATHY, FAMILIAL HYPERTROPHIC, 2; CMH2
CMM	GDB:119059	MELANOMA, MALIGNANT
COL11A1	GDB:120595	COLLAGEN, TYPE XI, ALPHA-1; COL11A1
COL9A2	GDB:138310	COLLAGEN, TYPE IX, ALPHA-2 CHAIN;

		COL9A2 EPIPHYSEAL DYSPLASIA, MULTIPLE, 2; EDM2
CPT2	GDB:127272	MYOPATHY WITH DEFICIENCY OF CARNITINE PALMITOYLTRANSFERASE II HYPOGLYCEMIA, HYPOKETOTIC, WITH DEFICIENCY OF CARNITINE PALMITOYLTRANSFERASE CARNITINE PALMITOYLTRANSFERASE II; CPT2
CRB1	GDB:333930	RETINITIS PIGMENTOSA-12; RP12
CSE	GDB:596182	CHOREOATHETOSIS/SPASTICITY, EPISODIC; CSE
CSF3R	GDB:126430	COLONY STIMULATING FACTOR 3 RECEPTOR, GRANULOCYTE; CSF3R
CTPA	GDB:9863168	CATARACT, POSTERIOR POLAR
CTSK	GDB:453910	PYCNODYSOSTOSIS CATHEPSIN K; CTSK
DBT	GDB:118784	MAPLE SYRUP URINE DISEASE, TYPE 2
DIO1	GDB:136449	THYROXINE DEIODINASE TYPE I; TXDI1
DISC1	GDB:9992707	DISORDER-2; SCZD2

DPYD	GDB:364102	DIHYDROPYRIMIDINE DEHYDROGENASE; DPYD
EKV	GDB:119106	ERYTHROKERATODERMIA VARIABILIS; EKV
ENO1	GDB:119871	PHOSPHOPYRUVATE HYDRATASE; PPH
ENO1P	GDB:135006	PHOSPHOPYRUVATE HYDRATASE; PPH
EPB41	GDB:119865	ERYTHROCYTE MEMBRANE PROTEIN BAND 4.1; EPB41 HEREDITARY HEMOLYTIC
EPHX1	GDB:119876	EPOXIDE HYDROLASE 1, MICROSOMAL; EPHX1
F13B	GDB:119893	FACTOR XIII, B SUBUNIT; F13B
F5	GDB:119896	FACTOR V DEFICIENCY
FCGR2A	GDB:119903	Fc FRAGMENT OF IgG, LOW AFFINITY IIa, RECEPTOR FOR; FCGR2A
FCGR2B	GDB:128183	Fc FRAGMENT OF IgG, LOW AFFINITY IIa, RECEPTOR FOR; FCGR2A
FCGR3A	GDB:119904	Fc FRAGMENT OF IgG, LOW AFFINITY IIIa, RECEPTOR FOR; FCGR3A
FCHL	GDB:9837503	HYPERLIPIDEMIA, COMBINED

FH	GDB:119133	FUMARATE HYDRATASE; FH LEIOMYOMATA, HEREDITARY MULTIPLE, OF SKIN
FMO3	GDB:135136	FLAVIN-CONTAINING MONOOXYGENASE 3; FMO3 TRIMETHYLAMINURIA
FMO4	GDB:127981	FLAVIN-CONTAINING MONOOXYGENASE 2; FMO2
FUCA1	GDB:119237	FUCOSIDOSIS
FY	GDB:119242	BLOOD GROUP--DUFFY SYSTEM; Fy
GALE	GDB:119245	GALACTOSE EPIMERASE DEFICIENCY
GBA	GDB:119262	GAUCHER DISEASE, TYPE I; GD I
GFND	GDB:9958222	GLOMERULAR NEPHRITIS, FAMILIAL, WITH FIBRONECTIN DEPOSITS
GJA8	GDB:696369	CATARACT, ZONULAR PULVERULENT 1; CZP1 GAP JUNCTION PROTEIN, ALPHA-8, 50-KD; GJA8
GJB3	GDB:127820	ERYTHROKERATODERMIA VARIABILIS; EKV DEAFNESS, AUTOSOMAL DOMINANT NONSYNDROMIC SENSORINEURAL, 2; DFNA2
GLC3B	GDB:3801939	GLAUCOMA 3, PRIMARY INFANTILE,

		B; GLC3B
HF1	GDB:120041	H FACTOR 1; HF1
HMGCL	GDB:138445	HYDROXYMETHYLGLUTARICACIDURIA; HMGCL
HPC1	GDB:5215209	PROSTATE CANCER; PRCA1 PROSTATE CANCER, HEREDITARY 1
HRD	GDB:9862254	HYPOPARATHYROIDISM WITH SHORT STATURE, MENTAL RETARDATION, AND SEIZURES
HRPT2	GDB:125253	HYPERPARATHYROIDISM, FAMILIAL PRIMARY, WITH MULTIPLE OSSIFYING JAW
HSD3B2	GDB:134044	ADRENAL HYPERPLASIA II
HSPG2	GDB:126372	HEPARAN SULFATE PROTEOGLYCAN OF BASEMENT MEMBRANE; HSPG2 MYOTONIC MYOPATHY, DWARFISM, CHONDRODYSTROPHY, AND OCULAR AND FACIAL
KCNQ4	GDB:439046	DEAFNESS, AUTOSOMAL DOMINANT NONSYNDROMIC SENSORINEURAL, 2; DFNA2
KCS	GDB:9848740	KENNY-CAFFEY SYNDROME, RECESSIVE FORM
KIF1B	GDB:128645	CHARCOT-MARIE-TOOTH DISEASE, NEURONAL TYPE, A; CMT2A

LAMB3	GDB:251820	LAMININ, BETA 3; LAMB3
LAMC2	GDB:136225	LAMININ, GAMMA 2; LAMC2 EPIDERMOLYSIS BULLOSA LETALIS
LGMD1B	GDB:231606	MUSCULAR DYSTROPHY, LIMB-GIRDLE, TYPE 1B; LGMD1B
LMNA	GDB:132146	LAMIN A/C; LMNA LIPODYSTROPHY, FAMILIAL PARTIAL, DUNNIGAN TYPE; LDP1
LOR	GDB:132049	LORICRIN; LOR
MCKD1	GDB:9859381	POLYCYSTIC KIDNEYS, MEDULLARY TYPE
MCL1	GDB:139137	MYELOID CELL LEUKEMIA 1; MCL1
MPZ	GDB:125266	HYPERTROPHIC NEUROPATHY OF DEJERINE-SOTTAS MYELIN PROTEIN ZERO; MPZ
MTHFR	GDB:370882	5,10-@METHYLENETETRAHYDROFOLATE REDUCTASE; MTHFR
MTR	GDB:119440	METHYLTETRAHYDROFOLATE:L-HOMOCYSTEINE S-METHYLTRANSFERASE; MTR
MUTYH	GDB:9315115	ADENOMATOUS POLYPOSIS OF THE COLON; APC

MYOC	GDB:5584221	GLAUCOMA 1, OPEN ANGLE; GLC1A MYOCILIN; MYOC
NB	GDB:9958705	NEUROBLASTOMA; NB
NCF2	GDB:120223	GRANULOMATOUS DISEASE, CHRONIC, AUTOSOMAL CYTOCHROME-b-POSITIVE FORM
NEM1	GDB:127387	NEMALINE MYOPATHY 1, AUTOSOMAL DOMINANT; NEM1
NPHS2	GDB:9955617	ARRHYTHMOGENIC RIGHT VENTRICULAR DYSPLASIA, FAMILIAL, 2; ARVD2
NPPA	GDB:118727	NATRIURETIC PEPTIDE PRECURSOR A; NPPA
NRAS	GDB:119457	ONCOGENE NRAS; NRAS; NRAS1
NTRK1	GDB:127897	ONCOGENE TRK NEUROTROPHIC TYROSINE KINASE, RECEPTOR, TYPE 1; NTRK1 NEUROPATHY, CONGENITAL SENSORY, WITH ANHIDROSIS
OPTA2	GDB:9955577	OSTEOPETROSIS, AUTOSOMAL DOMINANT, TYPE II; OPA2
PBX1	GDB:125351	PRE-B-CELL LEUKEMIA TRANSCRIPTION FACTOR-1; PBX1
PCHC	GDB:9955586	PHEOCHROMOCYTOMA
PGD	GDB:119486	6-@PHOSPHOGLUCONATE DEHYDROGENASE, ERYTHROCYTE

PHA2A	GDB:9955628	PSEUDOHYPOALDOSTERONISM, TYPE II; PHA2
PHGDH	GDB:9958261	3-@PHOSPHOGLYCERATE DEHYDROGENASE DEFICIENCY
PKLR	GDB:120294	PYRUVATE KINASE DEFICIENCY OF ERYTHROCYTE
PKP1	GDB:4249598	PLAKOPHILIN 1; PKP1
PLA2G2A	GDB:120296	PHOSPHOLIPASE A2, GROUP IIA; PLA2G2A
PLOD	GDB:127821	PROCOLLAGEN-LYSINE, 2-OXOGLUTARATE 5-DIOXYGENASE; PLOD EHLERS-DANLOS SYNDROME, TYPE VI; E-D VI; EDS VI
PPOX	GDB:118852	PROTOPORPHYRINOGEN OXIDASE; PPOX
PPT	GDB:125227	CEROID-LIPOFUSCINOSIS, NEURONAL 1, INFANTILE; CLN1 PALMITOYL-PROTEIN THIOESTERASE; PPT
PRCC	GDB:3888215	PAPILLARY RENAL CELL CARCINOMA; PRCC
PRG4	GDB:9955719	ARTHROPATHY-CAMPTODACTYLY SYNDROME
PSEN2	GDB:633044	ALZHEIMER DISEASE, FAMILIAL, TYPE 4; AD4

PTOS1	GDB:6279920	PTOSIS, HEREDITARY CONGENITAL 1; PTOS1
REN	GDB:120345	RENIN; REN
RFX5	GDB:6288464	REGULATORY FACTOR 5; RFX5
RHD	GDB:119551	RHESUS BLOOD GROUP, D ANTIGEN; RHD
RMD1	GDB:448902	RIPPLING MUSCLE DISEASE-1; RMD1
RPE65	GDB:226519	RETINAL PIGMENT EPITHELIUM-SPECIFIC PROTEIN, 65-KD; RPE65 AMAUROSIS CONGENITA OF LEBER II
SCCD	GDB:9955558	CORNEAL DYSTROPHY, CRYSTALLINE, OF SCHNYDER
SERPINC1	GDB:119024	ANTITHROMBIN III DEFICIENCY
SJS1	GDB:1381631	MYOTONIC MYOPATHY, DWARFISM, CHONDRODYSTROPHY, AND OCULAR AND FACIAL
SLC19A2	GDB:9837779	THIAMINE-RESPONSIVE MEGALOBLASTIC ANEMIA SYNDROME
SLC2A1	GDB:120627	SOLUTE CARRIER FAMILY 2, MEMBER 1; SLC2A1
SPTA1	GDB:119601	ELLIPTOCYTOSIS, RHESUS-UNLINKED TYPE HEREDITARY HEMOLYTIC SPECTRIN, ALPHA, ERYTHROCYTIC 1; SPTA1

TAL1	GDB:120759	T-CELL ACUTE LYMPHOCYTIC LEUKEMIA 1; TAL1
TNFSF6	GDB:422178	APOPTOSIS ANTIGEN LIGAND 1; APT1LG1
TNNT2	GDB:221879	TROPONIN-T2, CARDIAC; TNNT2
TPM3	GDB:127872	ONCOGENE TRK TROPOMYOSIN 3; TPM3
TSHB	GDB:120467	THYROID-STIMULATING HORMONE, BETA CHAIN; TSHB
UMPK	GDB:120481	URIDINE MONOPHOSPHATE KINASE; UMPK
UOX	GDB:127539	URATE OXIDASE; UOX
UROD	GDB:119628	PORPHYRIA CUTANEA TARDA; PCT
USH2A	GDB:120483	USHER SYNDROME, TYPE II; USH2
VMGLOM	GDB:9958134	GLOMUS TUMORS, MULTIPLE
VWS	GDB:120532	CLEFT LIP AND/OR PALATE WITH MUCOUS CYSTS OF LOWER LIP
WS2B	GDB:407579	WAARDENBURG SYNDROME, TYPE 2B; WS2B

Table 4: Genes, Locations and Genetic Disorders on Chromosome 2

Gene	**GDB Accession ID**	**Location**	**OMIM Link**

ABCB11	GDB:9864786	2q24-2q24 2q24.3-2q24.3	CHOLESTASIS, PROGRESSIVE FAMILIAL INTRAHEPATIC 2; PFIC2
ABCG5	GDB:10450298	2p21-2p21	PHYTOSTEROLEMIA
ABCG8	GDB:10450300	2p21-2p21	PHYTOSTEROLEMIA
ACADL	GDB:118745	2q34-2q35	ACYL-CoA DEHYDROGENASE, LONG-CHAIN, DEFICIENCY OF
ACP1	GDB:118962	2p25-2p25	PHOSPHATASE, ACID, OF ERYTHROCYTE; ACP1
AGXT	GDB:127113	2q37.3-2q37.3	OXALOSIS I
AHHR	GDB:118984	2pter-2q31	CYTOCHROME P450, SUBFAMILY I, POLYPEPTIDE 1; CYP1A1
ALMS1	GDB:9865539	2p13-2p12 2p14-2p13 2p13.1-2p13.1	ALSTROM SYNDROME
ALPP	GDB:119672	2q37.1-2q37.1	ALKALINE PHOSPHATASE, PLACENTAL; ALPP
ALS2	GDB:135696	2q33-2q35	AMYOTROPHIC LATERAL SCLEROSIS 2, JUVENILE; ALS2
APOB	GDB:119686	2p24-2p23 2p24-2p24	APOLIPOPROTEIN B; APOB
BDE	GDB:9955730	2q37-2q37	BRACHYDACTYLY, TYPE E; BDE
BDMR	GDB:533064	2q37-2q37	BRACHYDACTYLY-MENTAL

			RETARDATION SYNDROME; BDMR
BJS	GDB:9955717	2q34-2q36	TORTI AND NERVE DEAFNESS
BMPR2	GDB:642243	2q33-2q33 2q33-2q34	PULMONARY HYPERTENSION, PRIMARY; PPH1 BONE MORPHOGENETIC RECEPTOR TYPE II; BMPR2
CHRNA1	GDB:120586	2q24-2q32	CHOLINERGIC RECEPTOR, NICOTINIC, ALPHA POLYPEPTIDE 1; CHRNA1
CMCWTD	GDB:11498919	2p22.3-2p21	FAMILIAL CHRONIC MUCOCUTANEOUS, DOMINANT TYPE
CNGA3	GDB:434398	2q11.2-2q11.2	COLORBLINDNESS, TOTAL CYCLIC NUCLEOTIDE GATED CHANNEL, OLFACTORY, 3; CNG3
COL3A1	GDB:118729	2q31-2q32.3 2q32.2-2q32.2	COLLAGEN, TYPE III; COL3A1 EHLERS-DANLOS SYNDROME, TYPE IV, AUTOSOMAL DOMINANT
COL4A3	GDB:128351	2q36-2q37	COLLAGEN, TYPE IV, ALPHA-3 CHAIN; COL4A3
COL4A4	GDB:132673	2q35-2q37	COLLAGEN, TYPE IV, ALPHA-4 CHAIN; COL4A4
COL6A3	GDB:119066	2q37.3-2q37.3	COLLAGEN, TYPE VI, ALPHA-3 CHAIN; COL6A3 MYOPATHY, BENIGN CONGENITAL, WITH CONTRACTURES

CPS1	GDB:119799	2q33-2q36 2q34-2q35 2q35-2q35	HYPERAMMONEMIA DUE TO CARBAMOYLPHOSPHATE SYNTHETASE I DEFICIENCY
CRYGA	GDB:119076	2q33-2q35	CRYSTALLIN, GAMMA A; CRYGA
CRYGEP1	GDB:119808	2q33-2q35	CRYSTALLIN, GAMMA A; CRYGA
CYP1B1	GDB:353515	2p21-2p21 2p22-2p21 2pter-2qter	GLAUCOMA 3, PRIMARY INFANTILE, A; GLC3A CYTOCHROME P450, SUBFAMILY I (DIOXIN-INDUCIBLE), POLYPEPTIDE 1; CYP1B1
CYP27A1	GDB:128129	2q33-2qter	CEREBROTENDINOUS XANTHOMATOSIS
DBI	GDB:119837	2q12-2q21	DIAZEPAM BINDING INHIBITOR; DBI
DES	GDB:119841	2q35-2q35	DESMIN; DES
DYSF	GDB:340831	2p-2p 2p13-2p13 2pter-2p12	MUSCULAR DYSTROPHY, LIMB-GIRDLE, TYPE 2B; LGMD2B MUSCULAR DYSTROPHY, LATE-ONSET DISTAL
EDAR	GDB:9837372	2q11-2q13	DYSPLASIA, HYPOHIDROTIC ECTODERMAL DYSPLASIA, ANHIDROTIC
EFEMP1	GDB:1220111	2p16-2p16	DOYNE HONEYCOMB DEGENERATION OF RETINA FIBRILLIN-LIKE; FBNL

EIF2AK3	GDB:9956743	2p12-2p12	EPIPHYSEAL DYSPLASIA, MULTIPLE, WITH EARLY-ONSET DIABETES MELLITUS
ERCC3	GDB:119881	2q21-2q21	EXCISION-REPAIR, COMPLEMENTING DEFECTIVE, IN CHINESE HAMSTER, 3; ERCC3
FSHR	GDB:127510	2p21-2p16	FOLLICLE-STIMULATING HORMONE RECEPTOR; FSHR GONADAL DYSGENESIS, XX TYPE
GAD1	GDB:119244	2q31-2q31	PYRIDOXINE DEPENDENCY WITH SEIZURES
GINGF	GDB:9848875	2p21-2p21	GINGIVAL SON OF SEVENLESS (DROSOPHILA) HOMOLOG 1; SOS1
GLC1B	GDB:1297553	2q1-2q13	GLAUCOMA 1, OPEN ANGLE, B; GLC1B
GPD2	GDB:354558	2q24.1-2q24.1	GLYCEROL-3-PHOSPHATE DEHYDROGENASE-2; GPD2
GYPC	GDB:120027	2q14-2q21	BLOOD GROUP--GERBICH; Ge
HADHA	GDB:434026	2p23-2p23	HYDROXYACYL-CoA DEHYDROGENASE/3-KETOACYL-CoA THIOLASE/ENOYL-CoA HYDRATASE,
HADHB	GDB:344953	2p23-2p23	HYDROXYACYL-CoA DEHYDROGENASE/3-KETOACYL-CoA THIOLASE/ENOYL-CoA HYDRATASE,

HOXD13	GDB:127225	2q31-2q31	HOMEO BOX-D13; HOXD13 SYNDACTYLY, TYPE II
HPE2	GDB:136066	2p21-2p21	MIDLINE CLEFT SYNDROME
IGKC	GDB:120088	2p12-2p12 2p11.2-2p11.2	IMMUNOGLOBULIN KAPPA CONSTANT REGION; IGKC
IHH	GDB:511203	2q33-2q35 2q35-2q35 2pter-2qter	BRACHYDACTYLY, TYPE A1; BDA1 INDIAN HEDGEHOG, DROSOPHILA, HOMOLOG OF; IHH
IRS1	GDB:133974	2q36-2q36	INSULIN RECEPTOR SUBSTRATE 1; IRS1
ITGA6	GDB:128027	2pter-2qter	INTEGRIN, ALPHA-6; ITGA6
KHK	GDB:391903	2p23.3-2p23.2	FRUCTOSURIA
KYNU	GDB:9957925	2q22.2-2q23.3	
LCT	GDB:120140	2q21-2q21	DISACCHARIDE INTOLERANCE II
LHCGR	GDB:125260	2p21-2p21	LUTEINIZING HORMONE/CHORIOGONADOTROPIN RECEPTOR; LHCGR
LSFC	GDB:9956219	2-2 2p16-2p16	CYTOCHROME c OXIDASE DEFICIENCY, FRENCH-CANADIAN TYPE
MSH2	GDB:203983	2p16-2p16 2p22-2p21	COLON CANCER, FAMILIAL, NONPOLYPOSIS TYPE 1; FCC1
MSH6	GDB:632803	2p16-2p16	G/T MISMATCH-BINDING

			PROTEIN; GTBP
NEB	GDB:120224	2q24.1-2q24.2	NEBULIN; NEB NEMALINE MYOPATHY 2, AUTOSOMAL RECESSIVE; NEM2
NMTC	GDB:11498336	2q21-2q21	THYROID CARCINOMA, PAPILLARY
NPHP1	GDB:128050	2q13-2q13	NEPHRONOPHTHISIS, FAMILIAL JUVENILE 1; NPHP1
PAFAH1P1	GDB:435099	2p11.2-2p11.2	PLATELET-ACTIVATING FACTOR ACETYLHYDROLASE, GAMMA SUBUNIT
PAX3	GDB:120495	2q36-2q36 2q35-2q35	KLEIN-WAARDENBURG SYNDROME WAARDENBURG SYNDROME; WS1
PAX8	GDB:136447	2q12-2q14	PAIRED BOX HOMEOTIC GENE 8; PAX8
PMS1	GDB:386403	2q31-2q33	POSTMEIOTIC SEGREGATION INCREASED (S. CEREVISIAE)-1; PMS1
PNKD	GDB:5583973	2q33-2q35	CHOREOATHETOSIS, FAMILIAL PAROXYSMAL; FPD1
PPH1	GDB:1381541	2q31-2q32 2q33-2q33	PULMONARY HYPERTENSION, PRIMARY; PPH1
PROC	GDB:120317	2q13-2q21 2q13-2q14	PROTEIN C DEFICIENCY, CONGENITAL THROMBOTIC DISEASE DUE TO
REG1A	GDB:132455	2p12-2p12	REGENERATING

			ISLET-DERIVED 1-ALPHA; REG1A
SAG	GDB:120365	2q37.1-2q37.1	S-ANTIGEN; SAG
SFTPB	GDB:120374	2p12-2p11.2	SURFACTANT-ASSOCIATED PROTEIN, PULMONARY-3; SFTP3
SLC11A1	GDB:371444	2q35-2q35	CIRRHOSIS, PRIMARY; PBC NATURAL RESISTANCE-ASSOCIATED MACROPHAGE PROTEIN 1; NRAMP1
SLC3A1	GDB:202968	2p16.3-2p16.3 2p21-2p21	SOLUTE CARRIER FAMILY 3, MEMBER 1; SLC3A1 CYSTINURIA; CSNU
SOS1	GDB:230004	2p22-2p21	GINGIVAL SON OF SEVENLESS (DROSOPHILA) HOMOLOG 1; SOS1
SPG4	GDB:230127	2p24-2p21	SPASTIC PARAPLEGIA-4, AUTOSOMAL DOMINANT; SPG4
SRD5A2	GDB:127343	2p23-2p23	PSEUDOVAGINAL PERINEOSCROTAL HYPOSPADIAS; PPSH
TCL4	GDB:136378	2q34-2q34	T-CELL LEUKEMIA/LYMPHOMA-4; TCL4
TGFA	GDB:120435	2p13-2p13	TRANSFORMING GROWTH FACTOR, ALPHA; TGFA
TMD	GDB:9837196	2q31-2q31	TIBIAL MUSCULAR DYSTROPHY, TARDIVE

TPO	GDB:120446	2p25-2p25 2p25-2p24	THYROID HORMONOGENESIS, GENETIC DEFECT IN, IIA
UGT1	GDB:120007	2q37-2q37	UDP GLUCURONOSYLTRANSFERAS E 1 FAMILY, A1; UGT1A1
UV24	GDB:9955737	2pter-2qter	UV-DAMAGE, EXCISION REPAIR OF, UV-24
WSS	GDB:9955707	2q32-2q32	WRINKLY SKIN SYNDROME; WSS
XDH	GDB:266386	2p23-2p22	XANTHINURIA
ZAP70	GDB:433738	2q11-2q13 2q12-2q12	SYK-RELATED TYROSINE KINASE; SRK
ZFHX1B	GDB:9958310	2q22-2q22	DISEASE, MICROCEPHALY, AND IRIS COLOBOMA

Table 5: Genes, Locations and Genetic Disorders on Chromosome 3

Gene	**GDB Accession ID**	**Location**	**OMIM Link**
ACAA1	GDB:119643	3p23-3p22	PEROXISOMAL 3-OXOACYL-COENZYME A THIOLASE DEFICIENCY
AGTR1	GDB:132359	3q21-3q25	ANGIOTENSIN II RECEPTOR, VASCULAR TYPE 1; AT2R1
AHSG	GDB:118985	3q27-3q27	ALPHA-2-HS-GLYCOPROTEIN; AHSG
AMT	GDB:132138	3p21.3-3p21.2 3p21.2-3p21.1	HYPERGLYCINEMIA, ISOLATED NONKETOTIC, TYPE II; NKH2

ARP	GDB:9959049	3p21.1-3p21.1	ARGININE-RICH PROTEIN
BBS3	GDB:376501	3p-3p 3p12.3-3q11.1	BARDET-BIEDL SYNDROME, TYPE 3; BBS3
BCHE	GDB:120558	3q26.1-3q26.2	BUTYRYLCHOLINESTERASE; BCHE
BCPM	GDB:433809	3q21-3q21	BENIGN CHRONIC PEMPHIGUS; BCPM
BTD	GDB:309078	3p25-3p25	BIOTINIDASE; BTD
CASR	GDB:134196	3q21-3q24	HYPOCALCIURIC HYPERCALCEMIA, FAMILIAL; HHC1
CCR2	GDB:337364	3p21-3p21	CHEMOKINE (C-C) RECEPTOR 2; CMKBR2
CCR5	GDB:1230510	3p21-3p21	CHEMOKINE (C-C) RECEPTOR 5; CMKBR5
CDL1	GDB:136344	3q26.3-3q26.3	DE LANGE SYNDROME; CDL
CMT2B	GDB:604021	3q13-3q22	CHARCOT-MARIE-TOOTH DISEASE, NEURONAL TYPE, B; CMT2B
COL7A1	GDB:128750	3p21-3p21 3p21.3-3p21.3	COLLAGEN, TYPE VII, ALPHA-1; COL7A1
CP	GDB:119069	3q23-3q25 3q21-3q24	CERULOPLASMIN; CP
CRV	GDB:11498333	3p21.3-3p21.1	VASCULOPATHY, RETINAL, WITH CEREBRAL

			LEUKODYSTROPHY
CTNNB1	GDB:141922	3p22-3p22 3p21.3-3p21.3	CATENIN, BETA 1; CTNNB1
DEM	GDB:681157	3p12-3q11	DEMENTIA, FAMILIAL NONSPECIFIC; DEM
ETM1	GDB:9732523	3q13-3q13	TREMOR, HEREDITARY ESSENTIAL 1; ETM1
FANCD2	GDB:698345	3p25.3-3p25.3 3pter-3p24.2	FANCONI PANCYTOPENIA, COMPLEMENTATION GROUP D
FIH	GDB:9955790	3q13-3q13	HYPOPARATHYROIDISM, FAMILIAL ISOLATED; FIH
FOXL2	GDB:129025	3q23-3q23 3q22-3q23	BLEPHAROPHIMOSIS, EPICANTHUS INVERSUS, AND PTOSIS; BPES
GBE1	GDB:138442	3p12-3p12	GLYCOGEN STORAGE DISEASE IV
GLB1	GDB:119987	3p22-3p21.33 3p21.33-3p21.33	GANGLIOSIDOSIS, GENERALIZED GM1, TYPE I
GLC1C	GDB:3801941	3q21-3q24	GLAUCOMA 1, OPEN ANGLE, C; GLC1C
GNAI2	GDB:120516	3p21.3-3p21.2	GUANINE NUCLEOTIDE-BINDING PROTEIN, ALPHA-INHIBITING, POLYPEPTIDE-2;
GNAT1	GDB:119277	3p21.3-3p21.2	GUANINE NUCLEOTIDE-BINDING

			PROTEIN, ALPHA-TRANSDUCING, POLYPEPTIDE
GP9	GDB:126370	3pter-3qter	PLATELET GLYCOPROTEIN IX; GP9
GPX1	GDB:119282	3q11-3q12 3p21.3-3p21.3	GLUTATHIONE PEROXIDASE; GPX1
HGD	GDB:203935	3q21-3q23	ALKAPTONURIA; AKU
HRG	GDB:120055	3q27-3q27	HISTIDINE-RICH GLYCOPROTEIN; HRG; HRGP
ITIH1	GDB:120107	3p21.2-3p21.1	INTER-ALPHA-TRYPSIN INHIBITOR, HEAVY CHAIN-1; ITIH1; IATIH; ITIH
KNG	GDB:125256	3q27-3q27	FLAUJEAC FACTOR DEFICIENCY
LPP	GDB:1391795	3q27-3q28	LIM DOMAIN-CONTAINING PREFERRED TRANSLOCATION PARTNER IN LIPOMA; LPP
LRS1	GDB:682448	3p21.1-3p14.1	LARSEN SYNDROME, AUTOSOMAL DOMINANT; LRS1
MCCC1	GDB:135989	3q27-3q27 3q25-3q27	BETA-METHYLCROTONYLGLY CINURIA I
MDS1	GDB:250411	3q26-3q26	MYELODYSPLASIA SYNDROME 1; MDS1
MHS4	GDB:574245	3q13.1-3q13.1	HYPERTHERMIA SUSCEPTIBILITY-4; MHS4

MITF	GDB:214776	3p14.1-3p12	MICROPHTHALMIA-ASSOCIATED TRANSCRIPTION FACTOR; MITF WAARDENBURG SYNDROME, TYPE II; WS2
MLH1	GDB:249617	3p23-3p22 3p21.3-3p21.3	COLON CANCER, FAMILIAL, NONPOLYPOSIS TYPE 2; FCC2
MYL3	GDB:120218	3p21.3-3p21.2	MYOSIN, LIGHT CHAIN, ALKALI, VENTRICULAR AND SKELETAL SLOW; MYL3
MYMY	GDB:11500610	3p26-3p24.2	DISEASE
OPA1	GDB:118848	3q28-3q29	OPTIC ATROPHY 1; OPA1
PBXP1	GDB:125352	3q22-3q23	PRE-B-CELL LEUKEMIA TRANSCRIPTION FACTOR-1; PBX1
PCCB	GDB:119474	3q21-3q22	GLYCINEMIA, KETOTIC, II
POU1F1	GDB:129070	3p11-3p11	POU DOMAIN, CLASS 1, TRANSCRIPTION FACTOR 1; POU1F1
PPARG	GDB:1223810	3p25-3p25	CANCER OF COLON PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR, GAMMA; PPARG
PROS1	GDB:120721	3p11-3q11 3p11.1-3q11.2	PROTEIN S, ALPHA; PROS1
PTHR1	GDB:138128	3p22-3p21.1	METAPHYSEAL CHONDRODYSPLASIA, MURK JANSEN TYPE PARATHYROID HORMONE RECEPTOR 1; PTHR1

RCA1	GDB:230233	3p14.2-3p14.2	RENAL CARCINOMA, FAMILIAL, ASSOCIATED 1; RCA1
RHO	GDB:120347	3q21.3-3q24	RHODOPSIN; RHO
SCA7	GDB:454471	3p21.1-3p12	SPINOCEREBELLAR ATAXIA 7; SCA7
SCLC1	GDB:9955750	3p23-3p21	SMALL-CELL CANCER OF THE LUNG; SCCL
SCN5A	GDB:132152	3p21-3p21	SODIUM CHANNEL, VOLTAGE-GATED, TYPE V, ALPHA POLYPEPTIDE; SCN5A
SI	GDB:120377	3q25.2-3q26.2	DISACCHARIDE INTOLERANCE I
SLC25A20	GDB:6503297	3p21.31-3p21.31	CARNITINE-ACYLCARNITINE TRANSLOCASE; CACT
SLC2A2	GDB:119995	3q26.2-3q27 3q26.1-3q26.3	SOLUTE CARRIER FAMILY 2, MEMBER 2; SLC2A2 FANCONI-BICKEL SYNDROME; FBS
TF	GDB:120432	3q21-3q21	TRANSFERRIN; TF
TGFBR2	GDB:224909	3p22-3p22 3pter-3p24.2	TRANSFORMING GROWTH FACTOR-BETA RECEPTOR, TYPE II; TGFBR2
THPO	GDB:374007	3q26.3-3q27	THROMBOPOIETIN; THPO
THRB	GDB:120731	3p24.1-3p22 3p24.3-3p24.3	THYROID HORMONE RECEPTOR, BETA; THRB

TKT	GDB:132402	3p14.3-3p14.3	WERNICKE-KORSAKOFF SYNDROME
TM4SF1	GDB:250815	3q21-3q25	TUMOR-ASSOCIATED ANTIGEN L6; TAAL6
TRH	GDB:128072	3pter-3qter	THYROTROPIN-RELEASING HORMONE DEFICIENCY
UMPS	GDB:120482	3q13-3q13	OROTICACIDURIA I
UQCRC1	GDB:141850	3p21.3-3p21.2 3p21.3-3p21.3	UBIQUINOL-CYTOCHROME c REDUCTASE CORE PROTEIN I; UQCRC1
USH3A	GDB:392645	3q21-3q25	USHER SYNDROME, TYPE III; USH3
VHL	GDB:120488	3p26-3p25	VON HIPPEL-LINDAU SYNDROME; VHL
WS2A	GDB:128053	3p14.2-3p13	MICROPHTHALMIA-ASSOCIATE D TRANSCRIPTION FACTOR; MITF WAARDENBURG SYNDROME, TYPE II; WS2
XPC	GDB:134769	3p25.1-3p25.1	XERODERMA PIGMENTOSUM, COMPLEMENTATION GROUP C; XPC
ZNF35	GDB:120507	3p21-3p21	ZINC FINGER PROTEIN-35; ZNF35

Table 6: Genes, Locations and Genetic Disorders on Chromosome 4

Gene	**GDB Accession ID**	**Location**	**OMIM Link**

ADH1B	GDB:119651	4q21-4q23 4q22-4q22	ALCOHOL DEHYDROGENASE-2; ADH2
ADH1C	GDB:119652	4q21-4q23 4q22-4q22	ALCOHOL DEHYDROGENASE-3; ADH3
AFP	GDB:119660	4q11-4q13	ALPHA-FETOPROTEIN; AFP
AGA	GDB:118981	4q23-4q35 4q32-4q33	ASPARTYLGLUCOSAMINURIA ; AGU
AIH2	GDB:118751	4q11-4q13 4q13.3-4q21.2	AMELOGENESIS IMPERFECTA 2, HYPOPLASTIC LOCAL, AUTOSOMAL DOMINANT;
ALB	GDB:118990	4q11-4q13	ALBUMIN; ALB
ASMD	GDB:119705	4q-4q 4q28-4q31	ANTERIOR SEGMENT OCULAR DYSGENESIS; ASOD
BFHD	GDB:11498907	4q34.1-4q35	DYSPLASIA, BEUKES TYPE
CNGA1	GDB:127557	4p14-4q13	CYCLIC NUCLEOTIDE GATED CHANNEL, PHOTORECEPTOR, cGMP GATED, 1; CNCGi
CRBM	GDB:9958132	4p16.3-4p16.3	CHERUBISM
DCK	GDB:126810	4q13.3-4q21.1	DEOXYCYTIDINE KINASE; DCK
DFNA6	GDB:636175	4p16.3-4p16.3	DEAFNESS, AUTOSOMAL DOMINANT NONSYNDROMIC SENSORINEURAL, 6; DFNA6
DSPP	GDB:5560457	4pter-4qter 4q21.3-4q21.3	DENTIN PHOSPHOPROTEIN; DPP DENTINOGENESIS

			IMPERFECTA; DGI1
DTDP2	GDB:9955810	4q-4q	DENTIN DYSPLASIA, TYPE II
ELONG	GDB:11498700	4q24-4q24	
ENAM	GDB:9955259	4q21-4q21	AMELOGENESIS IMPERFECTA 2, HYPOPLASTIC LOCAL, AUTOSOMAL DOMINANT; AMELOGENESIS IMPERFECTA, HYPOPLASTIC TYPE
ETFDH	GDB:135992	4q32-4q35	GLUTARICACIDURIA IIC; GA IIC
EVC	GDB:555573	4p16-4p16	ELLIS-VAN CREVELD SYNDROME; EVC
F11	GDB:119891	4q35-4q35	PTA DEFICIENCY
FABP2	GDB:119127	4q28-4q31	FATTY ACID BINDING PROTEIN 2, INTESTINAL; FABP2
FGA	GDB:119129	4q28-4q28	AMYLOIDOSIS, FAMILIAL VISCERAL FIBRINOGEN, A ALPHA POLYPEPTIDE; FGA
FGB	GDB:119130	4q28-4q28	FIBRINOGEN, B BETA POLYPEPTIDE; FGB
FGFR3	GDB:127526	4p16.3-4p16.3	ACHONDROPLASIA; ACH BLADDER CANCER FIBROBLAST GROWTH FACTOR RECEPTOR-3; FGFR3
FGG	GDB:119132	4q28-4q28	FIBRINOGEN, G GAMMA POLYPEPTIDE; FGG

FSHMD1A	GDB:119914	4q35-4q35	FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY 1A; FSHMD1A
GC	GDB:119263	4q12-4q13 4q12-4q12	GROUP-SPECIFIC COMPONENT; GC
GNPTA	GDB:119280	4q21-4q23	MUCOLIPIDOSIS II; ML2; ML II
GNRHR	GDB:136456	4q13-4q13 4q21.2-4q21.2	GONADOTROPIN-RELEASING HORMONE RECEPTOR; GNRHR
GYPA	GDB:118890	4q28-4q31 4q28.2-4q31.1	BLOOD GROUP--MN LOCUS; MN
HCA	GDB:9954675	4q33-4qter	HYPERCALCIURIA, FAMILIAL IDIOPATHIC
HCL2	GDB:119305	4q28-4q31 4q-4q	HAIR COLOR-2; HCL2
HD	GDB:119307	4p16.3-4p16.3	HUNTINGTON DISEASE; HD
HTN3	GDB:125601	4q12-4q21	HISTATIN-3; HTN3
HVBS6	GDB:120687	4q32-4q32	HEPATOCELLULAR CARCINOMA-2; HCC2
IDUA	GDB:119327	4p16.3-4p16.3	MUCOPOLYSACCHARIDOSIS TYPE I; MPS I
IF	GDB:120077	4q24-4q25 4q25-4q25	COMPLEMENT COMPONENT-3 INACTIVATOR, DEFICIENCY OF
JPD	GDB:120113	4pter-4qter	PERIODONTITIS, JUVENILE;

		4q12-4q13	JPD
KIT	GDB:120117	4q12-4q12	V-KIT HARDY-ZUCKERMAN 4 FELINE SARCOMA VIRAL ONCOGENE HOMOLOG; KIT
KLKB1	GDB:127575	4q34-4q35 4q35-4q35	FLETCHER FACTOR DEFICIENCY
LQT4	GDB:682072	4q25-4q27	SYNDROME WITHOUT PSYCHOMOTOR RETARDATION
MANBA	GDB:125261	4q21-4q25	MANNOSIDOSIS, BETA; MANB1
MLLT2	GDB:136792	4q21-4q21	MYELOID/LYMPHOID OR MIXED LINEAGE LEUKEMIA, TRANSLOCATED TO, 2; MLLT2
MSX1	GDB:120683	4p16.3-4p16.1 4p16.1-4p16.1	MSH, DROSOPHILA, HOMEO BOX, HOMOLOG OF, 1; MSX1
MTP	GDB:228961	4q24-4q24	MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN, 88 KD; MTP
NR3C2	GDB:120188	4q31-4q31 4q31.1-4q31.1	PSEUDOHYPOALDOSTERONISM, TYPE I, AUTOSOMAL RECESSIVE; PHA1
PBT	GDB:120260	4q12-4q21	PIEBALD TRAIT; PBT
PDE6B	GDB:125915	4p16.3-4p16.3	NIGHTBLINDNESS, CONGENITAL STATIONARY; CSNB3 PHOSPHODIESTERASE 6B, cGMP-SPECIFIC, ROD, BETA; PDE6B

PEE1	GDB:7016765	4q31-4q34 4q25-4qter	1; PEE1
PITX2	GDB:134770	4q25-4q27 4q25-4q26 4q25-4q25	IRIDOGONIODYSGENESIS, TYPE 2; IRID2 RIEGER SYNDROME, TYPE 1; RIEG1 RIEG BICOID-RELATED HOMEOBOX TRANSCRIPTION FACTOR 1; RIEG1 HOMEO BOX 2
PKD2	GDB:118851	4q21-4q23	POLYCYSTIC KIDNEY DISEASE 2; PKD2
QDPR	GDB:120331	4p15.3-4p15.3 4p15.31-4p15.31	PHENYLKETONURIA II
SGCB	GDB:702072	4q12-4q12	MUSCULAR DYSTROPHY, LIMB-GIRDLE, TYPE 2E; LGMD2E
SLC25A4	GDB:119680	4q35-4q35	ADENINE NUCLEOTIDE TRANSLOCATOR 1; ANT1 PROGRESSIVE EXTERNAL OPHTHALMOPLEGIA; PEO
SNCA	GDB:439047	4q21.3-4q22 4q21-4q21	SYNUCLEIN, ALPHA; SNCA PARKINSON DISEASE, FAMILIAL, TYPE 1; PARK1
SOD3	GDB:125291	4p16.3-4q21	SUPEROXIDE DISMUTASE, EXTRACELLULAR; SOD3
STATH	GDB:120391	4q11-4q13	STATHERIN; STATH; STR
TAPVR1	GDB:392646	4p13-4q11	ANOMALOUS PULMONARY VENOUS RETURN; APVR

TYS	GDB:119624	4q-4q	SCLEROTYLOSIS; TYS
WBS2	GDB:132426	4q33-4q35.1	WILLIAMS-BEUREN SYNDROME; WBS
WFS1	GDB:434294	4p-4p 4p16-4p16	DIABETES MELLITUS AND INSIPIDUS WITH OPTIC ATROPHY AND DEAFNESS
WHCR	GDB:125355	4p16.3-4p16.3	WOLF-HIRSCHHORN SYNDROME; WHS

Table 7: Genes, Locations and Genetic Disorders on Chromosome 5

Gene	**GDB Accession ID**	**OMIM Link**
ADAMTS2	GDB:9957209	EHLERS-DANLOS SYNDROME, TYPE VII, AUTOSOMAL RECESSIVE
ADRB2	GDB:120541	BETA-2-ADRENERGIC RECEPTOR; ADRB2
AMCN	GDB:9836823	ARTHROGRYPOSIS MULTIPLEX CONGENITA, NEUROGENIC TYPE
AP3B1	GDB:9955590	HERMANSKY-PUDLAK SYNDROME; HPS
APC	GDB:119682	ADENOMATOUS POLYPOSIS OF THE COLON; APC
ARSB	GDB:119008	MUCOPOLYSACCHARIDOSIS TYPE VI; MPS VI
B4GALT7	GDB:9957653	SYNDROME, PROGEROID FORM

BHR1	GDB:9956078	ASTHMA
C6	GDB:119045	COMPLEMENT COMPONENT-6, DEFICIENCY OF
C7	GDB:119046	COMPLEMENT COMPONENT-7, DEFICIENCY OF
CCAL2	GDB:5584265	CHONDROCALCINOSIS, FAMILIAL ARTICULAR
CKN1	GDB:128586	COCKAYNE SYNDROME, TYPE I; CKN1
CMDJ	GDB:9595425	CRANIOMETAPHYSEAL DYSPLASIA, JACKSON TYPE; CMDJ
CRHBP	GDB:127438	CORTICOTROPIN RELEASING HORMONE-BINDING PROTEIN; CRHBP
CSF1R	GDB:120600	COLONY-STIMULATING FACTOR-1 RECEPTOR; CSF1R
DHFR	GDB:119845	DIHYDROFOLATE REDUCTASE; DHFR
DIAPH1	GDB:9835482	DEAFNESS, AUTOSOMAL DOMINANT NONSYNDROMIC SENSORINEURAL, 1; DFNA1 DIAPHANOUS, DROSOPHILA, HOMOLOG OF, 1
DTR	GDB:119853	DIPHTHERIA TOXIN SENSITIVITY; DTS
EOS	GDB:9956083	EOSINOPHILIA, FAMILIAL

ERVR	GDB:9835857	HYALOIDEORETINAL DEGENERATION OF WAGNER
F12	GDB:119892	HAGEMAN FACTOR DEFICIENCY
FBN2	GDB:128122	CONTRACTURAL ARACHNODACTYLY, CONGENITAL; CCA
GDNF	GDB:450609	GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR; GDNF
GHR	GDB:119984	GROWTH HORMONE RECEPTOR; GHR
GLRA1	GDB:118801	GLYCINE RECEPTOR, ALPHA-1 SUBUNIT; GLRA1 KOK DISEASE
GM2A	GDB:120000	TAY-SACHS DISEASE, AB VARIANT
HEXB	GDB:119308	SANDHOFF DISEASE
HSD17B4	GDB:385059	17-@BETA-HYDROXYSTEROID DEHYDROGENASE IV; HSD17B4
ITGA2	GDB:128031	INTEGRIN, ALPHA-2; ITGA2
KFS	GDB:9958987	VERTEBRAL FUSION
LGMD1A	GDB:118832	MUSCULAR DYSTROPHY, LIMB-GIRDLE, TYPE 1A; LGMD1A
LOX	GDB:119367	LYSYL OXIDASE; LOX
LTC4S	GDB:384080	LEUKOTRIENE C4 SYNTHASE; LTC4S

MAN2A1	GDB:136413	MANNOSIDASE, ALPHA, II; MANA2 DYSERYTHROPOIETIC ANEMIA, CONGENITAL, TYPE II
MCC	GDB:128163	MUTATED IN COLORECTAL CANCERS; MCC
MCCC2	GDB:135990	II
MSH3	GDB:641986	MutS, E. COLI, HOMOLOG OF, 3; MSH3
MSX2	GDB:138766	MSH (DROSOPHILA) HOMEO BOX HOMOLOG 2; MSX2 PARIETAL FORAMINA, SYMMETRIC; PFM
NR3C1	GDB:120017	GLUCOCORTICOID RECEPTOR; GRL
PCSK1	GDB:128033	PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 1; PCSK1
PDE6A	GDB:120265	PHOSPHODIESTERASE 6A, cGMP-SPECIFIC, ROD, ALPHA; PDE6A
PFBI	GDB:9956096	INTENSITY OF INFECTION IN
RASA1	GDB:120339	RAS p21 PROTEIN ACTIVATOR 1; RASA1
SCZD1	GDB:120370	DISORDER-1; SCZD1
SDHA	GDB:378037	SUCCINATE DEHYDROGENASE COMPLEX, SUBUNIT A, FLAVOPROTEIN; SDHA
SGCD	GDB:5886421	SARCOGLYCAN, DELTA; SGCD

SLC22A5	GDB:9863277	CARNITINE DEFICIENCY, SYSTEMIC, DUE TO DEFECT IN RENAL REABSORPTION
SLC26A2	GDB:125421	DIASTROPHIC DYSPLASIA; DTD EPIPHYSEAL DYSPLASIA, MULTIPLE; MED NEONATAL OSSEOUS DYSPLASIA I ACHONDROGENESIS, TYPE IB; ACG1B
SLC6A3	GDB:132445	SOLUTE CARRIER FAMILY 6, MEMBER 3; SLC6A3 DEFICIT-HYPERACTIVITY DISORDER; ADHD
SM1	GDB:9834488	SCHISTOSOMA MANSONI SUSCEPTIBILITY/RESISTANCE
SMA@	GDB:120378	SPINAL MUSCULAR ATROPHY I; SMA I SURVIVAL OF MOTOR NEURON 1, TELOMERIC; SMN1
SMN1	GDB:5215173	SPINAL MUSCULAR ATROPHY I; SMA I SURVIVAL OF MOTOR NEURON 1, TELOMERIC; SMN1
SMN2	GDB:5215175	SPINAL MUSCULAR ATROPHY I; SMA I SURVIVAL OF MOTOR NEURON 2, CENTROMERIC; SMN2
SPINK5	GDB:9956114	NETHERTON DISEASE
TCOF1	GDB:127390	TREACHER COLLINS-FRANCESCHETTI SYNDROME 1; TCOF1
TGFBI	GDB:597601	CORNEAL DYSTROPHY, GRANULAR TYPE CORNEAL DYSTROPHY, LATTICE TYPE I; CDL1

		TRANSFORMING GROWTH FACTOR, BETA-INDUCED, 68 KD; TGFBI

Table 8: Genes, Locations and Genetic Disorders on Chromosome 6

Gene	GDB Accession ID	OMIM Link
ALDH5A1	GDB:454767	SUCCINIC SEMIALDEHYDE DEHYDROGENASE, NAD(+)-DEPENDENT; SSADH
ARG1	GDB:119006	ARGININEMIA
AS	GDB:135697	ANKYLOSING SPONDYLITIS; AS
ASSP2	GDB:119017	CITRULLINEMIA
BCKDHB	GDB:118759	MAPLE SYRUP URINE DISEASE, TYPE IB
BF	GDB:119726	GLYCINE-RICH BETA-GLYCOPROTEIN; GBG
C2	GDB:119731	COMPLEMENT COMPONENT-2, DEFICIENCY OF
C4A	GDB:119732	COMPLEMENT COMPONENT 4A; C4A
CDKN1A	GDB:266550	CYCLIN-DEPENDENT KINASE INHIBITOR 1A; CDKN1A
COL10A1	GDB:128635	COLLAGEN, TYPE X, ALPHA 1; COL10A1
COL11A2	GDB:119788	COLLAGEN, TYPE XI, ALPHA-2; COL11A2 STICKLER SYNDROME, TYPE

		II; STL2 DEAFNESS, AUTOSOMAL DOMINANT NONSYNDROMIC SENSORINEURAL, 13; DFNA13
CYP21A2	GDB:120605	ADRENAL HYPERPLASIA, CONGENITAL, DUE TO 21-HYDROXYLASE DEFICIENCY
DYX2	GDB:437584	DYSLEXIA, SPECIFIC, 2; DYX2
EJM1	GDB:119864	MYOCLONIC EPILEPSY, JUVENILE; EJM1
ELOVL4	GDB:11499609	STARGARDT DISEASE 3; STGD3
EPM2A	GDB:3763331	EPILEPSY, PROGRESSIVE MYOCLONIC 2; EPM2
ESR1	GDB:119120	ESTROGEN RECEPTOR; ESR
EYA4	GDB:700062	DEAFNESS, AUTOSOMAL DOMINANT NONSYNDROMIC SENSORINEURAL, 10; DFNA10
F13A1	GDB:120614	FACTOR XIII, A1 SUBUNIT; F13A1
FANCE	GDB:1220236	FANCONI ANEMIA, COMPLEMENTATION GROUP E; FACE
GCLC	GDB:132915	GAMMA-GLUTAMYLCYSTEINE SYNTHETASE DEFICIENCY, HEMOLYTIC ANEMIA DUE
GJA1	GDB:125196	GAP JUNCTION PROTEIN, ALPHA-1, 43 KD; GJA1
GLYS1	GDB:136421	GLYCOSURIA, RENAL

GMPR	GDB:127058	GUANINE MONOPHOSPHATE REDUCTASE
GSE	GDB:9956235	DISEASE; CD
HCR	GDB:9993306	PSORIASIS, SUSCEPTIBILITY TO HFEGDB:119309 HEMOCHROMATOSIS; HFE
HLA-A	GDB:119310	HLA-A HISTOCOMPATIBILITY TYPE; HLAA HLA-DPB1GDB:120636 LA-DP HISTOCOMPATIBILITY TYPE, BETA-1 SUBUNIT
HLA-DRA	GDB:120641	HLA-DR HISTOCOMPATIBILITY TYPE; HLA-DRA
HPFH	GDB:9849006	HETEROCELLULAR HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN
ICS1	GDB:136433	IMMOTILE CILIA SYNDROME-1; ICS1
IDDM1	GDB:9953173	DIABETES MELLITUS, JUVENILE-ONSET INSULIN-DEPENDENT; IDDM
IFNGR1	GDB:120688	INTERFERON, GAMMA, RECEPTOR-1; IFNGR1
IGAD1	GDB:6929077	SELECTIVE DEFICIENCY OF
IGF2R	GDB:120083	INSULIN-LIKE GROWTH FACTOR 2 RECEPTOR; IGF2R
ISCW	GDB:9956158	SUPPRESSION; IS

LAMA2	GDB:132362	LAMININ, ALPHA 2; LAMA2
LAP	GDB:9958992	LARYNGEAL ADDUCTOR PARALYSIS; LAP
LCA5	GDB:11498764	AMAUROSIS CONGENITA OF LEBER I
LPA	GDB:120699	APOLIPOPROTEIN(a); LPA
MCDR1	GDB:131406	MACULAR DYSTROPHY, RETINAL, 1, NORTH CAROLINA TYPE; MCDR1
MOCS1	GDB:9862235	MOLYBDENUM COFACTOR DEFICIENCY
MUT	GDB:120204	METHYLMALONICACIDURIA DUE TO METHYLMALONIC CoA MUTASE DEFICIENCY
MYB	GDB:119441	V-MYB AVIAN MYELOBLASTOSIS VIRAL ONCOGENE HOMOLOG; MYB
NEU1	GDB:120230	NEURAMINIDASE DEFICIENCY
NKS1	GDB:128100	SUSCEPTIBILITY TO LYSIS BY ALLOREACTIVE NATURAL KILLER CELLS; EC1
NYS2	GDB:9848763	NYSTAGMUS, CONGENITAL
OA3	GDB:136429	ALBINISM, OCULAR, AUTOSOMAL RECESSIVE; OAR
ODDD	GDB:6392584	OCULODENTODIGITAL DYSPLASIA; ODDD

OFC1	GDB:120247	OROFACIAL CLEFT 1; OFC1
PARK2	GDB:6802742	PARKINSONISM, JUVENILE
PBCA	GDB:9956321	BETA CELL AGENESIS WITH NEONATAL DIABETES MELLITUS
PBCRA1	GDB:3763333	CHORIORETINAL ATROPHY, PROGRESSIVE BIFOCAL; CRAPB
PDB1	GDB:136349	DISEASE OF BONE; PDB
PEX3	GDB:9955507	ZELLWEGER SYNDROME; ZS
PEX6	GDB:5592414	ZELLWEGER SYNDROME; ZS PEROXIN-6; PEX6
PEX7	GDB:6155803	RHIZOMELIC CHONDRODYSPLASIA PUNCTATA; RCDP PEROXIN-7; PEX7
PKHD1	GDB:433910	POLYCYSTIC KIDNEY AND HEPATIC DISEASE-1; PKHD1
PLA2G7	GDB:9958829	PLATELET-ACTIVATING FACTOR ACETYLHYDROLASE, SUBUNIT
PLG	GDB:119498	PLASMINOGEN; PLG
POLH	GDB:6963323	PIGMENTOSUM WITH NORMAL DNA REPAIR RATES
PPAC	GDB:9956248	ARTHROPATHY, PROGRESSIVE PSEUDORHEUMATOID, OF CHILDHOOD

PSORS1	GDB:6381310	PSORIASIS, SUSCEPTIBILITY TO
PUJO	GDB:9956231	MULTICYSTIC RENAL DYSPLASIA, BILATERAL; MRD
RCD1	GDB:333929	RETINAL CONE DEGENERATION
RDS	GDB:118863	RETINAL DEGENERATION, SLOW; RDS
RHAG	GDB:136011	RHESUS BLOOD GROUP-ASSOCIATED GLYCOPROTEIN; RHAG RH-NULL, REGULATOR TYPE; RHN
RP14	GDB:433713	RETINITIS PIGMENTOSA-14; RP14 TUBBY-LIKE PROTEIN 1; TULP1
RUNX2	GDB:392082	CLEIDOCRANIAL DYSPLASIA; CCD CORE-BINDING FACTOR, RUNT DOMAIN, ALPHA SUBUNIT 1; CBFA1
RWS	GDB:9956195	SENSITIVITY
SCA1	GDB:119588	SPINOCEREBELLAR ATAXIA 1; SCA1
SCZD3	GDB:635974	DISORDER-3; SCZD3
SIASD	GDB:433552	SIALIC ACID STORAGE DISEASE; SIASD
SOD2	GDB:119597	SUPEROXIDE DISMUTASE 2, MITOCHONDRIAL; SOD2
ST8	GDB:6118456	OVARIAN TUMOR
TAP1	GDB:132668	TRANSPORTER 1, ABC; TAP1

TAP2	GDB:132669	TRANSPORTER 2, ABC; TAP2
TFAP2B	GDB:681506	DUCTUS ARTERIOSUS; PDA TRANSCRIPTION FACTOR AP-2 BETA; TFAP2B
TNDM	GDB:9956265	DIABETES MELLITUS, TRANSIENT NEONATAL
TNF	GDB:120441	TUMOR NECROSIS FACTOR; TNF
TPBG	GDB:125568	TROPHOBLAST GLYCOPROTEIN; TPBG; M6P1
TPMT	GDB:209025	THIOPURINE S-METHYLTRANSFERASE; TPMT
TULP1	GDB:6199353	TUBBY-LIKE PROTEIN 1; TULP1
WISP3	GDB:9957361	ARTHROPATHY, PROGRESSIVE PSEUDORHEUMATOID, OF CHILDHOOD

Table 9: Genes, Locations and Genetic Disorders on Chromosome 7

Gene	**GDB Accession ID**	**OMIM Link**
AASS	GDB:11502144	HYPERLYSINEMIA
ABCB1	GDB:120712	P-GLYCOPROTEIN-1; PGY1
ABCB4	GDB:120713	P-GLYCOPROTEIN-3; PGY3
ACHE	GDB:118746	ACETYLCHOLINESTERASE BLOOD GROUP--Yt SYSTEM; YT

AQP1	GDB:129082	AQUAPORIN-1; AQP1 BLOOD GROUP--COLTON; CO
ASL	GDB:119703	ARGININOSUCCINICACIDURIA
ASNS	GDB:119706	ASPARAGINE SYNTHETASE; ASNS; AS
AUTS1	GDB:9864226	DISORDER
BPGM	GDB:119039	DIPHOSPHOGLYCERATE MUTASE DEFICIENCY OF ERYTHROCYTE
C7orf2	GDB:10794644	ACHEIROPODY
CACNA2D1	GDB:132010	CALCIUM CHANNEL, VOLTAGE-DEPENDENT, L TYPE, ALPHA-2/DELTA SUBUNIT; MALIGNANT HYPERTHERMIA SUSCEPTIBILITY-3
CCM1	GDB:580824	CEREBRAL CAVERNOUS MALFORMATIONS 1; CCM1
CD36	GDB:138800	CD36 ANTIGEN; CD36
CFTR	GDB:120584	CYSTIC FIBROSIS; CF DEFERENS, CONGENITAL BILATERAL APLASIA OF; CBAVD; CAVD
CHORDOMA	GDB:11498328	
CLCN1	GDB:134688	CHLORIDE CHANNEL 1, SKELETAL MUSCLE; CLCN1
CMH6	GDB:9956392	CARDIOMYOPATHY, FAMILIAL HYPERTROPHIC, WITH WOLFF-PARKINSON-WHITE

CMT2D	GDB:9953232	CHARCOT-MARIE-TOOTH DISEASE, NEURONAL TYPE, D
COL1A2	GDB:119062	COLLAGEN, TYPE I, ALPHA-2 POLYPEPTIDE; COL1A2 OSTEOGENESIS IMPERFECTA TYPE I OSTEOGENESIS IMPERFECTA TYPE IV; OI4
CRS	GDB:119073	CRANIOSYNOSTOSIS, TYPE 1; CRS1
CYMD	GDB:366594	MACULAR EDEMA, CYSTOID
DFNA5	GDB:636174	DEAFNESS, AUTOSOMAL DOMINANT NONSYNDROMIC SENSORINEURAL, 5; DFNA5
DLD	GDB:120608	LIPOAMIDE DEHYDROGENASE DEFICIENCY, LACTIC ACIDOSIS DUE TO
DYT11	GDB:10013754	MYOCLONUS, HEREDITARY ESSENTIAL
EEC1	GDB:136338	ECTRODACTYLY, ECTODERMAL DYSPLASIA, AND CLEFT LIP/PALATE; EEC
ELN	GDB:119107	ELASTIN; ELN WILLIAMS-BEUREN SYNDROME; WBS
ETV1	GDB:335229	ETS VARIANT GENE 1; ETV1
FKBP6	GDB:9955215	WILLIAMS-BEUREN SYNDROME; WBS
GCK	GDB:127550	DIABETES MELLITUS, AUTOSOMAL DOMINANT, TYPE II GLUCOKINASE; GCK

GHRHR	GDB:138465	GROWTH HORMONE-RELEASING HORMONE RECEPTOR; GHRHR
GHS	GDB:9956363	MICROSOMIA WITH RADIAL DEFECTS
GLI3	GDB:119990	PALLISTER-HALL SYNDROME; PHS GLI-KRUPPEL FAMILY MEMBER 3; GLI3 POSTAXIAL POLYDACTYLY, TYPE A1 GREIG CEPHALOPOLYSYNDACTYLY SYNDROME; GCPS
GPDS1	GDB:9956410	GLAUCOMA, PIGMENT-DISPERSION TYPE
GUSB	GDB:120025	MUCOPOLYSACCHARIDOSIS TYPE VII
HADH	GDB:120033	HYDROXYACYL-CoA DEHYDROGENASE/3-KETOACYL-CoA THIOLASE/ENOYL-CoA HYDRATASE,
HLXB9	GDB:136411	HOMEO BOX GENE HB9; HLXB9 SACRAL AGENESIS, HEREDITARY, WITH PRESACRAL MASS, ANTERIOR MENINGOCELE,
HOXA13	GDB:120656	HOMEO BOX A13; HOXA13
HPFH2	GDB:128071	HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN, HETEROCELLULAR, INDIAN
HRX	GDB:9958999	HRX
IAB	GDB:11498909	ANEURYSM, INTRACRANIAL BERRY
IMMP2L	GDB:11499195	GILLES DE LA TOURETTE SYNDROME; GTS

KCNH2	GDB:138126	LONG QT SYNDROME, TYPE 2; LQT2
LAMB1	GDB:119357	LAMININ BETA 1; LAMB1
LEP	GDB:136420	LEPTIN; LEP
MET	GDB:120178	MET PROTO-ONCOGENE; MET
NCF1	GDB:120222	GRANULOMATOUS DISEASE, CHRONIC, AUTOSOMAL CYTOCHROME-b-POSITIVE FORM
NM	GDB:119454	NEUTROPHIL CHEMOTACTIC RESPONSE; NCR
OGDH	GDB:118847	ALPHA-KETOGLUTARATE DEHYDROGENASE DEFICIENCY
OPN1SW	GDB:119032	TRITANOPIA
PEX1	GDB:9787110	ZELLWEGER SYNDROME; ZS PEROXIN-1; PEX1
PGAM2	GDB:120280	PHOSPHOGLYCERATE MUTASE, DEFICIENCY OF M SUBUNIT OF
PMS2	GDB:386406	POSTMEIOTIC SEGREGATION INCREASED (S. CEREVISIAE)-2; PMS2
PON1	GDB:120308	PARAOXONASE 1; PON1
PPP1R3A	GDB:136797	PROTEIN PHOSPHATASE 1, REGULATORY (INHIBITOR) SUBUNIT 3; PPP1R3

PRSS1	GDB:119620	PANCREATITIS, HEREDITARY; PCTT PROTEASE, SERINE, 1; PRSS1
PTC	GDB:118744	PHENYLTHIOCARBAMIDE TASTING
PTPN12	GDB:136846	PROTEIN-TYROSINE PHOSPHATASE, NONRECEPTOR TYPE, 12; PTPN12
RP10	GDB:138786	RETINITIS PIGMENTOSA-10; RP10
RP9	GDB:333931	RETINITIS PIGMENTOSA-9; RP9
SERPINE1	GDB:120297	PLASMINOGEN ACTIVATOR INHIBITOR, TYPE I; PAI1
SGCE	GDB:9958714	MYOCLONUS, HEREDITARY ESSENTIAL
SHFM1	GDB:128195	SPLIT-HAND/FOOT DEFORMITY, TYPE I; SHFD1
SHH	GDB:456309	HOLOPROSENCEPHALY, TYPE 3; HPE3 SONIC HEDGEHOG, DROSOPHILA, HOMOLOG OF; SHH
SLC26A3	GDB:138165	DOWN-REGULATED IN ADENOMA; DRA CHLORIDE DIARRHEA, FAMILIAL; CLD
SLC26A4	GDB:5584511	PENDRED SYNDROME; PDS DEAFNESS, NEUROSENSORY, AUTOSOMAL RECESSIVE, 4; DFNB4
SLOS	GDB:385950	SMITH-LEMLI-OPITZ SYNDROME
SMAD1	GDB:3763345	SPINAL MUSCULAR ATROPHY, DISTAL, WITH UPPER LIMB

		PREDOMINANCE; SMAD1
TBXAS1	GDB:128744	THROMBOXANE A SYNTHASE 1; TBXAS1
TWIST	GDB:135694	ACROCEPHALOSYNDACTYLY TYPE III TWIST, DROSOPHILA, HOMOLOG OF; TWIST
ZWS1	GDB:120511	ZELLWEGER SYNDROME; ZS

Table 10: Genes, Locations and Genetic Disorders on Chromosome 8

Gene	**GDB AccessionID**	**OMIM Link**
ACHM3	GDB:9120558	PINGELAPESE BLINDNESS
ADRB3	GDB:203869	BETA-3-ADRENERGIC RECEPTOR; ADRB3
ANK1	GDB:118737	SPHEROCYTOSIS, HEREDITARY; HS
CA1	GDB:119047	CARBONIC ANHYDRASE I, ERYTHROCYTE, ELECTROPHORETIC VARIANTS OF; CA1
CA2	GDB:119739	OSTEOPETROSIS WITH RENAL TUBULAR ACIDOSIS
CCAL1	GDB:512892	CHONDROCALCINOSIS WITH EARLY-ONSET OSTEOARTHRITIS; CCAL2
CLN8	GDB:252118	EPILEPSY, PROGRESSIVE, WITH MENTAL RETARDATION; EPMR
CMT4A	GDB:138755	CHARCOT-MARIE-TOOTH NEUROPATHY 4A; CMT4A

CNGB3	GDB:9993286	PINGELAPESE BLINDNESS
COH1	GDB:252122	COHEN SYNDROME; COH1
CPP	GDB:119798	CERULOPLASMIN; CP
CRH	GDB:119804	CORTICOTROPIN-RELEASING HORMONE; CRH
CYP11B1	GDB:120603	ADRENAL HYPERPLASIA, CONGENITAL, DUE TO 11-@BETA-HYDROXYLASE DEFICIENCY
CYP11B2	GDB:120514	CYTOCHROME P450, SUBFAMILY XIB, POLYPEPTIDE 2; CYP11B2
DECR1	GDB:453934	2,4-@DIENOYL-CoA REDUCTASE; DECR
DPYS	GDB:5885803	DIHYDROPYRIMIDINASE; DPYS
DURS1	GDB:9958126	DUANE SYNDROME
EBS1	GDB:119856	EPIDERMOLYSIS BULLOSA SIMPLEX, OGNA TYPE
ECA1	GDB:10796318	JUVENILE ABSENCE
EGI	GDB:128830	EPILEPSY, GENERALIZED, IDIOPATHIC; EGI
EXT1	GDB:135994	EXOSTOSES, MULTIPLE, TYPE I; EXT1 CHONDROSARCOMA
EYA1	GDB:5215167	BRANCHIOOTORENAL DYSPLASIA EYES ABSENT 1; EYA1

FGFR1	GDB:119913	ACROCEPHALOSYNDACTYLY TYPE V FIBROBLAST GROWTH FACTOR RECEPTOR-1; FGFR1
GNRH1	GDB:133746	GONADOTROPIN-RELEASING HORMONE 1; GNRH1 FAMILIAL HYPOGONADOTROPHIC
GSR	GDB:119288	GLUTATHIONE REDUCTASE; GSR
GULOP	GDB:128078	SCURVY
HR	GDB:595499	ALOPECIA UNIVERSALIS ATRICHIA WITH PAPULAR LESIONS HAIRLESS, MOUSE, HOMOLOG OF
KCNQ3	GDB:9787230	CONVULSIONS, BENIGN FAMILIAL NEONATAL, TYPE 2; BFNC2 POTASSIUM CHANNEL, VOLTAGE-GATED, SUBFAMILY Q, MEMBER 3
KFM	GDB:265291	KLIPPEL-FEIL SYNDROME; KFS; KFM
KWE	GDB:9315120	KERATOLYTIC WINTER ERYTHEMA
LGCR	GDB:120698	LANGER-GIEDION SYNDROME; LGS
LPL	GDB:120700	HYPERLIPOPROTEINEMIA, TYPE I
MCPH1	GDB:9834525	MICROCEPHALY; MCT
MOS	GDB:119396	TRANSFORMATION GENE: ONCOGENE MOS
MYC	GDB:120208	TRANSFORMATION GENE: ONCOGENE MYC; MYC

NAT1	GDB:125364	ARYLAMIDE ACETYLASE 1; AAC1
NAT2	GDB:125365	ISONIAZID INACTIVATION
NBS1	GDB:9598211	NIJMEGEN BREAKAGE SYNDROME
PLAT	GDB:119496	PLASMINOGEN ACTIVATOR, TISSUE; PLAT
PLEC1	GDB:4119073	EPIDERMOLYSIS BULLOSA SIMPLEX AND LIMB-GIRDLE MUSCULAR DYSTROPHY PLECTIN 1; PLEC1
PRKDC	GDB:234702	SEVERE COMBINED IMMUNODEFICIENCY DISEASE-1; SCID1 PROTEIN KINASE, DNA-ACTIVATED, CATALYTIC SUBUNIT; PRKDC
PXMP3	GDB:131487	PEROXIN-2; PEX2 ZELLWEGER SYNDROME; ZS
RP1	GDB:120352	RETINITIS PIGMENTOSA-1; RP1
SCZD6	GDB:9864736	DISORDER-2; SCZD2
SFTPC	GDB:120373	PULMONARY SURFACTANT APOPROTEIN PSP-C
SGM1	GDB:135350	KLIPPEL-FEIL SYNDROME; KFS; KFM
SPG5A	GDB:250332	SPASTIC PARAPLEGIA-5A, AUTOSOMAL RECESSIVE; SPG5A
STAR	GDB:635457	STEROIDOGENIC ACUTE REGULATORY PROTEIN; STAR

TG	GDB:120434	THYROGLOBULIN; TG
TRPS1	GDB:594960	TRICHORHINOPHALANGEAL SYNDROME, TYPE I; TRPS1
TTPA	GDB:512364	VITAMIN E, FAMILIAL ISOLATED DEFICIENCY OF; VED TOCOPHEROL (ALPHA) TRANSFER PROTEIN; TTPA
VMD1	GDB:119631	MACULAR DYSTROPHY, ATYPICAL VITELLIFORM; VMD1
WRN	GDB:128446	WERNER SYNDROME; WRN

Table 11: Genes, Locations and Genetic Disorders on Chromosome 9

Gene	**GDB AccessionID**	**OMIM Link**
ABCA1	GDB:305294	ANALPHALIPOPROTEINEMIA ATP-BINDING CASSETTE 1; ABC1
ABL1	GDB:119640	ABELSON MURINE LEUKEMIA VIRAL ONCOGENE HOMOLOG 1; ABL1
ABO	GDB:118956	ABO BLOOD GROUP; ABO
ADAMTS13	GDB:9956467	THROMBOCYTOPENIC PURPURA
AK1	GDB:119664	ADENYLATE KINASE-1; AK1
ALAD	GDB:119665	DELTA-AMINOLEVULINATE DEHYDRATASE; ALAD
ALDH1A1	GDB:119667	ALDEHYDE DEHYDROGENASE-1; ALDH1

ALDOB	GDB:119669	FRUCTOSE INTOLERANCE, HEREDITARY
AMBP	GDB:120696	PROTEIN HC; HCP
AMCD1	GDB:437519	ARTHROGRYPOSIS MULTIPLEX CONGENITA, DISTAL, TYPE 1; AMCD1
ASS	GDB:119010	CITRULLINEMIA
BDMF	GDB:9954424	BONE DYSPLASIA WITH MEDULLARY FIBROSARCOMA
BSCL	GDB:9957720	SEIP SYNDROME
C5	GDB:119734	COMPLEMENT COMPONENT-5, DEFICIENCY OF
CDKN2A	GDB:335362	MELANOMA, CUTANEOUS MALIGNANT, 2; CMM2 CYCLIN-DEPENDENT KINASE INHIBITOR 2A; CDKN2A
CHAC	GDB:6268491	CHOREOACANTHOCYTOSIS; CHAC
CHH	GDB:138268	CARTILAGE-HAIR HYPOPLASIA; CHH
CMD1B	GDB:677147	CARDIOMYOPATHY, DILATED 1B; CMD1B
COL5A1	GDB:131457	COLLAGEN, TYPE V, ALPHA-1 POLYPEPTIDE; COL5A1
CRAT	GDB:359759	CARNITINE ACETYLTRANSFERASE; CRAT
DBH	GDB:119836	DOPAMINE BETA-HYDROXYLASE, PLASMA; DBH

DFNB11	GDB:1220180	DEAFNESS, NEUROSENSORY, AUTOSOMAL RECESSIVE, 7; DFNB7
DFNB7	GDB:636178	DEAFNESS, NEUROSENSORY, AUTOSOMAL RECESSIVE, 7; DFNB7
DNAI1	GDB:11500297	IMMOTILE CILIA SYNDROME-1; ICS1
DYS	GDB:137085	DYSAUTONOMIA, FAMILIAL; DYS
DYT1	GDB:119854	DYSTONIA 1, TORSION; DYT1
ENG	GDB:137193	ENDOGLIN; ENG
EPB72	GDB:128993	ERYTHROCYTE SURFACE PROTEIN BAND 7.2; EPB72 STOMATOCYTOSIS I
FANCC	GDB:132672	FANCONI ANEMIA, COMPLEMENTATION GROUP C; FACC
FBP1	GDB:141539	FRUCTOSE-1,6-BISPHOPHATASE 1; FBP1
FCMD	GDB:250412	FUKUYAMA-TYPE CONGENITAL MUSCULAR DYSTROPHY; FCMD
FRDA	GDB:119951	FRIEDREICH ATAXIA 1; FRDA1
GALT	GDB:119971	GALACTOSEMIA
GLDC	GDB:128611	HYPERGLYCINEMIA, ISOLATED NONKETOTIC, TYPE I; NKH1
GNE	GDB:9954891	INCLUSION BODY MYOPATHY; IBM2

GSM1	GDB:9784210	GENIOSPASM 1; GSM1
GSN	GDB:120019	AMYLOIDOSIS V GELSOLIN; GSN
HSD17B3	GDB:347487	PSEUDOHERMAPHRODITISM, MALE, WITH GYNECOMASTIA
HSN1	GDB:3853677	NEUROPATHY, HEREDITARY SENSORY, TYPE 1
IBM2	GDB:3801447	INCLUSION BODY MYOPATHY; IBM2
LALL	GDB:9954426	LEUKEMIA, ACUTE, WITH LYMPHOMATOUS FEATURES; LALL
LCCS	GDB:386141	LETHAL CONGENITAL CONTRACTURE SYNDROME; LCCS
LGMD2H	GDB:9862233	DYSTROPHY, HUTTERITE TYPE
LMX1B	GDB:9834526	NAIL-PATELLA SYNDROME; NPS1
MLLT3	GDB:138172	MYELOID/LYMPHOID OR MIXED LINEAGE LEUKEMIA, TRANSLOCATED TO, 3; MLLT3
MROS	GDB:9954430	MELKERSSON SYNDROME
MSSE	GDB:128019	EPITHELIOMA, SELF-HEALING SQUAMOUS
NOTCH1	GDB:131400	NOTCH, DROSOPHILA, HOMOLOG OF, 1; NOTCH1
ORM1	GDB:120250	OROSOMUCOID 1; ORM1

PAPPA	GDB:134729	PREGNANCY-ASSOCIATED PLASMA PROTEIN A; PAPPA
PIP5K1B	GDB:686238	FRIEDREICH ATAXIA 1; FRDA1
PTCH	GDB:119447	BASAL CELL NEVUS SYNDROME; BCNS PATCHED, DROSOPHILA, HOMOLOG OF; PTCH
PTGS1	GDB:128070	PROSTAGLANDIN-ENDOPEROXIDASE SYNTHASE 1; PTGS1
RLN1	GDB:119552	RELAXIN; RLN1
RLN2	GDB:119553	RELAXIN, OVARIAN, OF PREGNANCY
RMRP	GDB:120348	MITOCHONDRIAL RNA-PROCESSING ENDORIBONUCLEASE, RNA COMPONENT OF; RMRP; CARTILAGE-HAIR HYPOPLASIA; CHH
ROR2	GDB:136454	BRACHYDACTYLY, TYPE B; BDB ROBINOW SYNDROME, RECESSIVE FORM NEUROTROPHIC TYROSINE KINASE, RECEPTOR-RELATED 2; NTRKR2
RPD1	GDB:9954440	RETINITIS PIGMENTOSA-DEAFNESS SYNDROME 1, AUTOSOMAL DOMINANT
SARDH	GDB:9835149	SARCOSINEMIA
TDFA	GDB:9954420	FACTOR, AUTOSOMAL
TEK	GDB:344185	VENOUS MALFORMATIONS, MULTIPLE CUTANEOUS AND MUCOSAL; VMCM TEK TYROSINE KINASE, ENDOTHELIAL; TEK

TSC1	GDB:120735	TUBEROUS SCLEROSIS-1; TSC1
TYRP1	GDB:126337	TYROSINASE-RELATED PROTEIN 1; TYRP1 ALBINISM III XANTHISM
XPA	GDB:125363	XERODERMA PIGMENTOSUM I

Table 12: Genes, Locations and Genetic Disorders on Chromosomes 10

Gene	**GDB Accession ID**	**OMIM Link**
CACNB2	GDB:132014	CALCIUM CHANNEL, VOLTAGE-DEPENDENT, BETA-2 SUBUNIT; CACNB2
COL17A1	GDB:131396	COLLAGEN, TYPE XVII, ALPHA-1 POLYPEPTIDE; COL17A1
CUBN	GDB:636049	MEGALOBLASTIC ANEMIA 1; MGA1
CYP17	GDB:119829	ADRENAL HYPERPLASIA, CONGENITAL, DUE TO 17-ALPHA-HYDROXYLASE DEFICIENCY
CYP2C19	GDB:119831	CYTOCHROME P450, SUBFAMILY IIC, POLYPEPTIDE 19; CYP2C19
CYP2C9	GDB:131455	CYTOCHROME P450, SUBFAMILY IIC, POLYPEPTIDE 9; CYP2C9
EGR2	GDB:120611	EARLY GROWTH RESPONSE-2; EGR2
EMX2	GDB:277886	EMPTY SPIRACLES, DROSOPHILA, 2, HOMOLOG OF; EMX2

EPT	GDB:9786112	EPILEPSY, PARTIAL; EPT
ERCC6	GDB:119882	EXCISION-REPAIR CROSS-COMPLEMENTING RODENT REPAIR DEFICIENCY, COMPLEMENTATION
FGFR2	GDB:127273	ACROCEPHALOSYNDACTYLY TYPE V FIBROBLAST GROWTH FACTOR RECEPTOR-2; FGFR2
HK1	GDB:120044	HEXOKINASE-1; HK1
HOX11	GDB:119607	HOMEO BOX-11; HOX11
HPS	GDB:127359	HERMANSKY-PUDLAK SYNDROME; HPS
IL2RA	GDB:119345	INTERLEUKIN-2 RECEPTOR, ALPHA; IL2RA
LGI1	GDB:9864936	EPILEPSY, PARTIAL; EPT
LIPA	GDB:120153	WOLMAN DISEASE
MAT1A	GDB:129077	METHIONINE ADENOSYLTRANSFERASE DEFICIENCY
MBL2	GDB:120167	MANNOSE-BINDING PROTEIN, SERUM; MBP1
MKI67	GDB:120185	PROLIFERATION-RELATED Ki-67 ANTIGEN; MKI67
MXI1	GDB:137182	MAX INTERACTING PROTEIN 1; MXI1

OAT	GDB:120246	ORNITHINE AMINOTRANSFERASE DEFICIENCY
OATL3	GDB:215803	ORNITHINE AMINOTRANSFERASE DEFICIENCY
PAX2	GDB:138771	PAIRED BOX HOMEOTIC GENE 2; PAX2
PCBD	GDB:138478	PTERIN-4-ALPHA-CARBINOLAMINE DEHYDRATASE; PCBD PRIMAPTERINURIA
PEO1	GDB:632784	PROGRESSIVE EXTERNAL OPHTHALMOPLEGIA; PEO
PHYH	GDB:9263423	REFSUM DISEASE PHYTANOYL-CoA HYDROXYLASE; PHYH
PNLIP	GDB:127916	LIPASE, CONGENITAL ABSENCE OF PANCREATIC
PSAP	GDB:120366	PROSAPOSIN; PSAP
PTEN	GDB:6022948	MACROCEPHALY, MULTIPLE LIPOMAS AND HEMANGIOMATA MULTIPLE HAMARTOMA SYNDROME; MHAM POLYPOSIS, JUVENILE INTESTINAL PHOSPHATASE AND TENSIN HOMOLOG; PTEN
RBP4	GDB:120342	RETINOL-BINDING PROTEIN, PLASMA; RBP4
RDPA	GDB:9954445	REFSUM DISEASE WITH INCREASED PIPECOLICACIDEMIA; RDPA

RET	GDB:120346	RET PROTO-ONCOGENE; RET
SDF1	GDB:433267	STROMAL CELL-DERIVED FACTOR 1; SDF1
SFTPA1	GDB:119593	PULMONARY SURFACTANT APOPROTEIN PSP-A; PSAP
SFTPD	GDB:132674	PULMONARY SURFACTANT APOPROTEIN PSP-D; PSP-D
SHFM3	GDB:386030	SPLIT-HAND/FOOT MALFORMATION, TYPE 3; SHFM3
SIAL	GDB:6549924	NEURAMINIDASE DEFICIENCY
THC2	GDB:10794765	THROMBOCYTOPENIA
TNFRSF6	GDB:132671	APOPTOSIS ANTIGEN 1; APT1
UFS	GDB:6380714	UROFACIAL SYNDROME; UFS
UROS	GDB:128112	PORPHYRIA, CONGENITAL ERYTHROPOIETIC; CEP

Table 13: Genes, Locations and Genetic Disorders on Chromosome 11

Gene	**GDB Accession ID**	**OMIM Link**
AA	GDB:568984	ATROPHIA AREATA; AA
ABCC8	GDB:591370	SULFONYLUREA RECEPTOR; SUR PERSISTENT HYPERINSULINEMIC HYPOGLYCEMIA OF INFANCY

ACAT1	GDB:126861	ALPHA-METHYLACETOACETICACIDURIA
ALX4	GDB:10450304	PARIETAL FORAMINA, SYMMETRIC; PFM
AMPD3	GDB:136013	ADENOSINE MONOPHOSPHATE DEAMINASE-3; AMPD3
ANC	GDB:9954484	CANAL CARCINOMA
APOA1	GDB:119684	AMYLOIDOSIS, FAMILIAL VISCERAL APOLIPOPROTEIN A-I OF HIGH DENSITY LIPOPROTEIN; APOA1
APOA4	GDB:119000	APOLIPOPROTEIN A-IV; APOA4
APOC3	GDB:119001	APOLIPOPROTEIN C-III; APOC3
ATM	GDB:593364	ATAXIA-TELANGIECTASIA; AT
BSCL2	GDB:9963996	SEIP SYNDROME
BWS	GDB:120567	BECKWITH-WIEDEMANN SYNDROME; BWS
CALCA	GDB:120571	CALCITONIN/CALCITONIN-RELATED POLYPEPTIDE, ALPHA; CALCA
CAT	GDB:119049	CATALASE; CAT
CCND1	GDB:128222	LEUKEMIA, CHRONIC LYMPHATIC; CLL CYCLIN D1; CCND1
CD3E	GDB:119764	CD3E ANTIGEN, EPSILON POLYPEPTIDE; CD3E

CD3G	GDB:119765	T3 T-CELL ANTIGEN, GAMMA CHAIN; T3G; CD3G
CD59	GDB:119769	CD59 ANTIGEN P18-20; CD59 HUMAN LEUKOCYTE ANTIGEN MIC11; MIC11
CDKN1C	GDB:593296	CYCLIN-DEPENDENT KINASE INHIBITOR 1C; CDKN1C
CLN2	GDB:125228	CEROID-LIPOFUSCINOSIS, NEURONAL 2, LATE INFANTILE TYPE; CLN2
CNTF	GDB:125919	CILIARY NEUROTROPHIC FACTOR; CNTF
CPT1A	GDB:597642	HYPOGLYCEMIA, HYPOKETOTIC, WITH DEFICIENCY OF CARNITINE PALMITOYLTRANSFERASE CARNITINE PALMITOYLTRANSFERASE I, LIVER; CPT1A
CTSC	GDB:642234	KERATOSIS PALMOPLANTARIS WITH PERIODONTOPATHIA KERATOSIS PALMOPLANTARIS WITH PERIODONTOPATHIA AND ONYCHOGRYPOSIS CATHEPSIN C; CTSC
DDB1	GDB:595014	DNA DAMAGE-BINDING PROTEIN; DDB1
DDB2	GDB:595015	DNA DAMAGE-BINDING PROTEIN-2; DDB2
DHCR7	GDB:9835302	SMITH-LEMLI-OPITZ SYNDROME
DLAT	GDB:118785	CIRRHOSIS, PRIMARY; PBC
DRD4	GDB:127782	DOPAMINE RECEPTOR D4; DRD4
ECB2	GDB:9958955	POLYCYTHEMIA, BENIGN FAMILIAL

ED4	GDB:9837373	DYSPLASIA, MARGARITA TYPE
EVR1	GDB:134029	EXUDATIVE VITREORETINOPATHY, FAMILIAL; EVR EXT2GDB:344921EXOSTOSES, MULTIPLE, TYPE II; EXT2 CHONDROSARCOMA
F2	GDB:119894	COAGULATION FACTOR II; F2
FSHB	GDB:119955	FOLLICLE-STIMULATING HORMONE, BETA POLYPEPTIDE; FSHB
FTH1	GDB:120617	FERRITIN HEAVY CHAIN 1; FTH1
GIF	GDB:118800	PERNICIOUS ANEMIA, CONGENITAL, DUE TO DEFECT OF INTRINSIC FACTOR
GSD1B	GDB:9837619	GLYCOGEN STORAGE DISEASE Ib
GSD1C	GDB:9837637	STORAGE DISEASE Ic
HBB	GDB:119297	HEMOGLOBIN--BETA LOCUS; HBB
HBBP1	GDB:120035	HEMOGLOBIN--BETA LOCUS; HBB
HBD	GDB:119298	HEMOGLOBIN--DELTA LOCUS; HBD
HBE1	GDB:119299	HEMOGLOBIN--EPSILON LOCUS; HBE1
HBG1	GDB:119300	HEMOGLOBIN, GAMMA A; HBG1
HBG2	GDB:119301	HEMOGLOBIN, GAMMA G; HBG2
HMBS	GDB:120528	PORPHYRIA, ACUTE INTERMITTENT; AIP

HND	GDB:9954478	HARTNUP DISORDER
HOMG2	GDB:9956484	MAGNESIUM WASTING, RENAL
HRAS	GDB:120684	BLADDER CANCER V-HA-RAS HARVEY RAT SARCOMA VIRAL ONCOGENE HOMOLOG; HRAS
HVBS1	GDB:120069	CANCER, HEPATOCELLULAR
IDDM2	GDB:128530	DIABETES MELLITUS, INSULIN-DEPENDENT, 2 DIABETES MELLITUS, JUVENILE-ONSET INSULIN-DEPENDENT; IDDM
IGER	GDB:119696	IgE RESPONSIVENESS, ATOPIC; IGER
INS	GDB:119349	INSULIN; INS
JBS	GDB:120111	JACOBSEN SYNDROME; JBS
KCNJ11	GDB:7009893	POTASSIUM CHANNEL, INWARDLY-RECTIFYING, SUBFAMILY J, MEMBER 11; KCNJ11 PERSISTENT HYPERINSULINEMIC HYPOGLYCEMIA OF INFANCY
KCNJ1	GDB:204206	POTASSIUM CHANNEL, INWARDLY-RECTIFYING, SUBFAMILY J, MEMBER 1; KCNJ1
KCNQ1	GDB:741244	LONG QT SYNDROME, TYPE 1; LQT1
LDHA	GDB:120141	LACTATE DEHYDROGENASE-A; LDHA
LRP5	GDB:9836818	OSTEOPOROSIS-PSEUDOGLIOMA SYNDROME; OPPG HIGH BONE MASS

MEN1	GDB:120173	MULTIPLE ENDOCRINE NEOPLASIA, TYPE 1; MEN1
MLL	GDB:128819	MYELOID/LYMPHOID OR MIXED-LINEAGE LEUKEMIA; MLL
MTACR1	GDB:125743	MULTIPLE TUMOR ASSOCIATED CHROMOSOME REGION 1; MTACR1
MYBPC3	GDB:579615	CARDIOMYOPATHY, FAMILIAL HYPERTROPHIC, 4; CMH4 MYOSIN-BINDING PROTEIN C, CARDIAC; MYBPC3
MYO7A	GDB:132543	MYOSIN VIIA; MYO7A DEAFNESS, NEUROSENSORY, AUTOSOMAL RECESSIVE, 2; DFNB2 DEAFNESS, AUTOSOMAL DOMINANT NONSYNDROMIC SENSORINEURAL, 11; DFNA11
NNO1	GDB:10450513	SIMPLE, AUTOSOMAL DOMINANT
OPPG	GDB:3789438	OSTEOPOROSIS-PSEUDOGLIOMA SYNDROME; OPPG
OPTB1	GDB:9954474	OSTEOPETROSIS, AUTOSOMAL RECESSIVE
PAX6	GDB:118997	PAIRED BOX HOMEOTIC GENE 6; PAX6
PC	GDB:119472	PYRUVATE CARBOXYLASE DEFICIENCY
PDX1	GDB:9836634	PYRUVATE DEHYDROGENASE COMPLEX, COMPONENT X
PGL2	GDB:511177	PARAGANGLIOMAS, FAMILIAL NONCHROMAFFIN, 2; PGL2

PGR	GDB:119493	PROGESTERONE RESISTANCE
PORC	GDB:128610	PORPHYRIA, CHESTER TYPE; PORC
PTH	GDB:119522	PARATHYROID HORMONE; PTH
PTS	GDB:118856	6-@PYRUVOYLTETRAHYDROPTERIN SYNTHASE; PTS
PVRL1	GDB:583951	ECTODERMAL DYSPLASIA, CLEFT LIP AND PALATE, HAND AND FOOT DEFORMITY, DYSPLASIA, MARGARITA TYPE POLIOVIRUS RECEPTOR RELATED; PVRR
PYGM	GDB:120329	GLYCOGEN STORAGE DISEASE V
RAG1	GDB:120334	RECOMBINATION ACTIVATING GENE-1; RAG1
RAG2	GDB:125186	RECOMBINATION ACTIVATING GENE-2; RAG2
ROM1	GDB:120350	ROD OUTER SEGMENT PROTEIN-1; ROM1
SAA1	GDB:120364	SERUM AMYLOID A1; SAA1
SCA5	GDB:378219	SPINOCEREBELLAR ATAXIA 5; SCA5
SCZD2	GDB:118874	DISORDER-2; SCZD2
SDHD	GDB:132456	PARAGANGLIOMAS, FAMILIAL NONCHROMAFFIN, 1; PGL1
SERPING1	GDB:119041	ANGIONEUROTIC EDEMA, HEREDITARY; HANE

SMPD1	GDB:128144	NIEMANN-PICK DISEASE
TCIRG1	GDB:9956269	OSTEOPETROSIS, AUTOSOMAL RECESSIVE
TCL2	GDB:9954468	LEUKEMIA, ACUTE T-CELL; ATL
TECTA	GDB:6837718	DEAFNESS, AUTOSOMAL DOMINANT NONSYNDROMIC SENSORINEURAL, 8; DFNA8 DEAFNESS, AUTOSOMAL DOMINANT NONSYNDROMIC SENSORINEURAL, 12; DFNA12
TH	GDB:119612	TYROSINE HYDROXYLASE; TH
TREH	GDB:9958953	TREHALASE
TSG101	GDB:1313414	TUMOR SUSCEPTIBILITY GENE 101; TSG101
TYR	GDB:120476	ALBINISM I
USH1C	GDB:132544	USHER SYNDROME, TYPE IC; USH1C
VMD2	GDB:133795	VITELLIFORM MACULAR DYSTROPHY; VMD2
VRNI	GDB:135662	VITREORETINOPATHY, NEOVASCULAR INFLAMMATORY; VRNI
WT1	GDB:120496	FRASIER SYNDROME WILMS TUMOR; WT1
WT2	GDB:118886	MULTIPLE TUMOR ASSOCIATED CHROMOSOME REGION 1; MTACR1
ZNF145	GDB:230064	PROMYELOCYTIC LEUKEMIA ZINC FINGER; PLZF

Table 14: Genes, Locations and Genetic Disorders on Chromosome 12

Gene	GDB Accession ID	OMIM Link
A2M	GDB:119639	ALPHA-2-MACROGLOBULIN; A2M
AAAS	GDB:9954498	GLUCOCORTICOID DEFICIENCY AND ACHALASIA
ACADS	GDB:118959	ACYL-CoA DEHYDROGENASE, SHORT-CHAIN; ACADS
ACLS	GDB:136346	ACROCALLOSAL SYNDROME; ACLS
ACVRL1	GDB:230240	OSLER-RENDU-WEBER SYNDROME 2; ORW2 ACTIVIN A RECEPTOR, TYPE II-LIKE KINASE 1; ACVRL1
ADHR	GDB:9954488	VITAMIN D-RESISTANT RICKETS, AUTOSOMAL DOMINANT
ALDH2	GDB:119668	ALDEHYDE DEHYDROGENASE-2; ALDH2
AMHR2	GDB:696210	ANTI-MULLERIAN HORMONE TYPE II RECEPTOR; AMHR2
AOM	GDB:118998	STICKLER SYNDROME, TYPE I; STL1
AQP2	GDB:141853	AQUAPORIN-2; AQP2 DIABETES INSIPIDUS, RENAL TYPE DIABETES INSIPIDUS, RENAL TYPE, AUTOSOMAL RECESSIVE
ATD	GDB:696353	ASPHYXIATING THORACIC DYSTROPHY; ATD

ATP2A2	GDB:119717	ATPase, Ca(2+)-TRANSPORTING, SLOW-TWITCH; ATP2A2 DARIER-WHITE DISEASE; DAR
BDC	GDB:5584359	BRACHYDACTYLY, TYPE C; BDC
C1R	GDB:119729	COMPLEMENT COMPONENT-C1r, DEFICIENCY OF
CD4	GDB:119767	T-CELL ANTIGEN T4/LEU3; CD4
CDK4	GDB:204022	CYCLIN-DEPENDENT KINASE 4; CDK4
CNA1	GDB:252119	CORNEA PLANA 1; CNA1
COL2A1	GDB:119063	STICKLER SYNDROME, TYPE I; STL1 COLLAGEN, TYPE II, ALPHA-1 CHAIN; COL2A1 ACHONDROGENESIS, TYPE II; ACG2
CYP27B1	GDB:9835730	PSEUDOVITAMIN D DEFICIENCY RICKETS; PDDR
DRPLA	GDB:270336	DENTATORUBRAL-PALLIDOLUYSIAN ATROPHY; DRPLA
ENUR2	GDB:666422	ENURESIS, NOCTURNAL, 2; ENUR2
FEOM1	GDB:345037	FIBROSIS OF EXTRAOCULAR MUSCLES, CONGENITAL; FEOM
FPF	GDB:9848880	PERIODIC FEVER, AUTOSOMAL DOMINANT
GNB3	GDB:120005	GUANINE NUCLEOTIDE-BINDING PROTEIN, BETA POLYPEPTIDE-3; GNB3

GNS	GDB:120006	MUCOPOLYSACCHARIDOSIS TYPE IIID
HAL	GDB:120746	HISTIDINEMIA
HBP1	GDB:701889	BRACHYDACTYLY WITH HYPERTENSION
HMGIC	GDB:362658	HIGH MOBILITY GROUP PROTEIN ISOFORM I-C; HMGIC
HMN2	GDB:9954508	MUSCULAR ATROPHY, ADULT SPINAL
HPD	GDB:135978	TYROSINEMIA, TYPE III
IGF1	GDB:120081	INSULINLIKE GROWTH FACTOR 1; IGF1
KCNA1	GDB:127903	POTASSIUM VOLTAGE-GATED CHANNEL, SHAKER-RELATED SUBFAMILY, MEMBER
KERA	GDB:252121	CORNEA PLANA 2; CNA2
KRAS2	GDB:120120	V-KI-RAS2 KIRSTEN RAT SARCOMA 2 VIRAL ONCOGENE HOMOLOG; KRAS2
KRT1	GDB:128198	KERATIN 1; KRT1
KRT2A	GDB:407640	ICHTHYOSIS, BULLOUS TYPE KERATIN 2A; KRT2A
KRT3	GDB:136276	KERATIN 3; KRT3

KRT4	GDB:120697	KERATIN 4; KRT4
KRT5	GDB:128110	EPIDERMOLYSIS BULLOSA HERPETIFORMIS, DOWLING-MEARA TYPE KERATIN 5; KRT5
KRT6A	GDB:128111	KERATIN 6A; KRT6A
KRT6B	GDB:128113	KERATIN 6B; KRT6B PACHYONYCHIA CONGENITA, JACKSON-LAWLER TYPE
KRTHB6	GDB:702078	MONILETHRIX KERATIN, HAIR BASIC (TYPE II) 6
LDHB	GDB:120147	LACTATE DEHYDROGENASE-B; LDHB
LYZ	GDB:120160	AMYLOIDOSIS, FAMILIAL VISCERAL LYSOZYME; LYZ
MGCT	GDB:9954504	TESTICULAR TUMORS
MPE	GDB:120191	MALIGNANT PROLIFERATION OF
MVK	GDB:134189	MEVALONICACIDURIA
MYL2	GDB:128829	MYOSIN, LIGHT CHAIN, REGULATORY VENTRICULAR; MYL2
NS1	GDB:439388	NOONAN SYNDROME 1; NS1
OAP	GDB:120245	OSTEOARTHROSIS, PRECOCIOUS; OAP

PAH	GDB:119470	PHENYLKETONURIA; PKU1
PPKB	GDB:696352	PALMOPLANTAR KERATODERMA, BOTHNIAN TYPE; PPKB
PRB3	GDB:119513	PAROTID SALIVARY GLYCOPROTEIN; G1
PXR1	GDB:433739	ZELLWEGER SYNDROME; ZS PEROXISOME RECEPTOR 1; PXR1
RLS	GDB:11501392	ACROMELALGIA, HEREDITARY
RSN	GDB:139158	RESTIN; RSN
SAS	GDB:128054	SARCOMA AMPLIFIED SEQUENCE; SAS
SCA2	GDB:128034	SPINOCEREBELLAR ATAXIA 2; SCA2 ATAXIN-2; ATX2
SCNN1A	GDB:366596	SODIUM CHANNEL, NONVOLTAGE-GATED, 1; SCNN1A
SMAL	GDB:9954506	SPINAL MUSCULAR ATROPHY, CONGENITAL NONPROGRESSIVE, OF LOWER LIMBS
SPPM	GDB:9954502	SCAPULOPERONEAL MYOPATHY; SPM
SPSMA	GDB:9954510	SCAPULOPERONEAL AMYOTROPHY, NEUROGENIC, NEW ENGLAND TYPE
TBX3	GDB:681969	ULNAR-MAMMARY SYNDROME; UMS T-BOX 3; TBX3

TBX5	GDB:6175917	HOLT-ORAM SYNDROME; HOS T-BOX 5; TBX5
TCF1	GDB:125297	TRANSCRIPTION FACTOR 1, HEPATIC; TCF1 MATURITY-ONSET DIABETES OF THE YOUNG, TYPE III; MODY3
TPI1	GDB:119617	TRIOSEPHOSPHATE ISOMERASE 1; TPI1
TSC3	GDB:127930	SCLEROSIS-3; TSC3
ULR	GDB:594089	UTERINE
VDR	GDB:120487	VITAMIN D-RESISTANT RICKETS WITH END-ORGAN UNRESPONSIVENESS TO 1,25-DIHYDROXYCHOLECALCIFEROL VITAMIN D RECEPTOR; VDR
VWF	GDB:119125	VON WILLEBRAND DISEASE; VWD

Table 15: Genes, Locations and Genetic Disorders on Chromosome 13

Gene	**GDB Accession ID**	**OMIM Link**
ATP7B	GDB:120494	WILSON DISEASE; WND
BRCA2	GDB:387848	BREAST CANCER 2, EARLY-ONSET; BRCA2
BRCD1	GDB:9954522	BREAST CANCER, DUCTAL, 1; BRCD1
CLN5	GDB:230991	CEROID-LIPOFUSCNOSIS, NEURONAL 5; CLN5

CPB2	GDB:129546	CARBOXYPEPTIDASE B2, PLASMA; CPB2
ED2	GDB:9834522	ECTODERMAL DYSPLASIA, HIDROTIC; HED
EDNRB	GDB:129075	ENDOTHELIN-B RECEPTOR; EDNRB HIRSCHSPRUNG DISEASE-2; HSCR2
ENUR1	GDB:594516	ENURESIS, NOCTURNAL, 1; ENUR1
ERCC5	GDB:120515	EXCISION-REPAIR, COMPLEMENTING DEFECTIVE, IN CHINESE HAMSTER, 5; ERCC5
F10	GDB:119890	X, QUANTITATIVE VARIATION IN FACTOR X DEFICIENCY; F10
F7	GDB:119897	FACTOR VII DEFICIENCY
GJB2	GDB:125247	GAP JUNCTION PROTEIN, BETA-2, 26 KD; GJB2 DEAFNESS, NEUROSENSORY, AUTOSOMAL RECESSIVE, 1; DFNB1 DEAFNESS, AUTOSOMAL DOMINANT NONSYNDROMIC SENSORINEURAL, 3; DFNA3
GJB6	GDB:9958357	ECTODERMAL DYSPLASIA, HIDROTIC; HED DEAFNESS, AUTOSOMAL DOMINANT NONSYNDROMIC SENSORINEURAL, 3; DFNA3
IPF1	GDB:448899	INSULIN PROMOTER FACTOR 1; IPF1
MBS1	GDB:128365	MOEBIUS SYNDROME; MBS
MCOR	GDB:9954520	CONGENITAL

PCCA	GDB:119473	GLYCINEMIA, KETOTIC, I
RB1	GDB:118734	BLADDER CANCER RETINOBLASTOMA; RB1
RHOK	GDB:371598	RHODOPSIN KINASE; RHOK
SCZD7	GDB:9864734	DISORDER-2; SCZD2
SGCG	GDB:3763329	MUSCULAR DYSTROPHY, LIMB GIRDLE, TYPE 2C; LGMD2C
SLC10A2	GDB:677534	SOLUTE CARRIER FAMILY 10, MEMBER 2; SLC10A2
SLC25A15	GDB:120042	HYPERORNITHINEMIA-HYPERAMMONEMIA-HOMOCITRULLINURIA SYNDROME
STARP1	GDB:635459	STEROIDOGENIC ACUTE REGULATORY PROTEIN; STAR
ZNF198	GDB:6382650	ZINC FINGER PROTEIN-198; ZNF198

Table 16: Genes, Locations and Genetic Disorders on Chromosome 14

Gene	**GDB Accession ID**	**OMIM Link**
ACHM1	GDB:132458	COLORBLINDNESS, TOTAL
ARVD1	GDB:371339	ARRHYTHMOGENIC RIGHT VENTRICULAR DYSPLASIA, FAMILIAL, 1; ARVD1
CTAA1	GDB:265299	CATARACT, ANTERIOR POLAR 1; CTAA1
DAD1	GDB:407505	DEFENDER AGAINST CELL DEATH; DAD1

DFNB5	GDB:636176	DEAFNESS, NEUROSENSORY, AUTOSOMAL RECESSIVE, 5; DFNB5
EML1	GDB:6328385	USHER SYNDROME, TYPE IA; USH1A
GALC	GDB:119970	KRABBE DISEASE
GCH1	GDB:118798	DYSTONIA, PROGRESSIVE, WITH DIURNAL VARIATION GTP CYCLOHYDROLASE I DEFICIENCY GTP CYCLOHYDROLASE I; GCH1
HE1	GDB:9957680	MALFORMATIONS, MULTIPLE, WITH LIMB ABNORMALITIES AND HYPOPITUITARISM
IBGC1	GDB:10450404	CEREBRAL CALCIFICATION, NONARTERIOSCLEROTIC
IGH@	GDB:118731	IgA CONSTANT HEAVY CHAIN 1; IGHA1 IMMUNOGLOBULIN: D (DIVERSITY) REGION OF HEAVY CHAIN IgA CONSTANT HEAVY CHAIN 2; IGHA2 IMMUNOGLOBULIN: J (JOINING) LOCI OF HEAVY CHAIN; IGHJ IMMUNOGLOBULIN: HEAVY Mu CHAIN; Mu1; IGHM1 IMMUNOGLOBULIN: VARIABLE REGION OF HEAVY CHAINS--Hv1; IGHV IgG HEAVY CHAIN LOCUS; IGHG1 IMMUNOGLOBULIN Gm-2; IGHG2 IMMUNOGLOBULIN Gm-3; IGHG3 IMMUNOGLOBULIN Gm-4; IGHG4 IMMUNOGLOBULIN: HEAVY DELTA CHAIN; IGHD IMMUNOGLOBULIN: HEAVY EPSILON CHAIN; IGHE
IGHC group	GDB:9992632	IgA CONSTANT HEAVY CHAIN 1; IGHA1 IgA CONSTANT HEAVY CHAIN 2; IGHA2 IMMUNOGLOBULIN: HEAVY Mu CHAIN; Mu1; IGHM1 IgG HEAVY CHAIN LOCUS; IGHG1 IMMUNOGLOBULIN Gm-2; IGHG2 IMMUNOGLOBULIN Gm-3; IGHG3 IMMUNOGLOBULIN Gm-4; IGHG4 IMMUNOGLOBULIN: HEAVY DELTA CHAIN;

		IGHD IMMUNOGLOBULIN: HEAVY EPSILON CHAIN; IGHE
IGHG1	GDB:120085	IgG HEAVY CHAIN LOCUS; IGHG1
IGHM	GDB:120086	IMMUNOGLOBULIN: HEAVY Mu CHAIN; Mu1; IGHM1
IGHR	GDB:9954529	G1(A1) SYNDROME
IV	GDB:139274	INVERSUS VISCERUM
LTBP2	GDB:453890	LATENT TRANSFORMING GROWTH FACTOR-BETA BINDING PROTEIN 2; LTBP2
MCOP	GDB:9954527	MICROPHTHALMOS
MJD	GDB:118840	MACHADO-JOSEPH DISEASE; MJD
MNG1	GDB:6540062	GOITER, MULTINODULAR 1; MNG1
MPD1	GDB:230271	MYOPATHY, LATE DISTAL HEREDITARY
MPS3C	GDB:9954532	MUCOPOLYSACCHARIDOSIS TYPE IIIC
MYH6	GDB:120214	MYOSIN, HEAVY POLYPEPTIDE 6; MYH6
MYH7	GDB:120215	MYOSIN, CARDIAC, HEAVY CHAIN, BETA; MYH7
NP	GDB:120239	NUCLEOSIDE PHOSPHORYLASE; NP
PABPN1	GDB:567135	OCULOPHARYNGEAL MUSCULAR DYSTROPHY; OPMD OCULOPHARYNGEAL

		MUSCULAR DYSTROPHY, AUTOSOMAL RECESSIVE POLYADENYLATE-BINDING PROTEIN-2; PABP2
PSEN1	GDB:135682	ALZHEIMER DISEASE, FAMILIAL, TYPE 3; AD3
PYGL	GDB:120328	GLYCOGEN STORAGE DISEASE VI
RPGRIP1	GDB:11498766	AMAUROSIS CONGENITA OF LEBER I
SERPINA1	GDB:120289	PROTEASE INHIBITOR 1; PI
SERPINA3	GDB:118955	ALPHA-1-ANTICHYMOTRYPSIN; AACT
SERPINA6	GDB:127865	CORTICOSTEROID-BINDING GLOBULIN; CBG
SLC7A7	GDB:9863033	DIBASICAMINOACIDURIA II
SPG3A	GDB:230126	SPASTIC PARAPLEGIA-3, AUTOSOMAL DOMINANT; SPG3A
SPTB	GDB:119602	ELLIPTOCYTOSIS, RHESUS-UNLINKED TYPE HEREDITARY HEMOLYTIC SPECTRIN, BETA, ERYTHROCYTIC; SPTB
TCL1A	GDB:250785	T-CELL LYMPHOMA OR LEUKEMIA
TCRAV17S1	GDB:642130	T-CELL ANTIGEN RECEPTOR, ALPHA SUBUNIT; TCRA
TCRAV5S1	GDB:451966	T-CELL ANTIGEN RECEPTOR, ALPHA SUBUNIT; TCRA

TGM1	GDB:125299	TRANSGLUTAMINASE 1; TGM1 ICHTHYOSIS CONGENITA
TITF1	GDB:132588	THYROID TRANSCRIPTION FACTOR 1; TITF1
TMIP	GDB:9954523	AND ULNA, DUPLICATION OF, WITH ABSENCE OF TIBIA AND RADIUS
TRA@	GDB:120404	T-CELL ANTIGEN RECEPTOR, ALPHA SUBUNIT; TCRA
TSHR	GDB:125313	THYROTROPIN, UNRESPONSIVENESS TO
USH1A	GDB:118885	USHER SYNDROME, TYPE IA; USH1A
VP	GDB:120492	PORPHYRIA VARIEGATA

Table 17: Genes, Locations and Genetic Disroders on Chromosome 15

Gene	**GDB Accession ID**	**OMIM Link**
ACCPN	GDB:5457725	CORPUS CALLOSUM, AGENESIS OF, WITH NEURONOPATHY
AHO2	GDB:9954535	HEREDITARY OSTEODYSTROPHY-2; AHO2
ANCR	GDB:119678	ANGELMAN SYNDROME
B2M	GDB:119028	BETA-2-MICROGLOBULIN; B2M
BBS4	GDB:511199	BARDET-BIEDL SYNDROME, TYPE 4; BBS4
BLM	GDB:135698	BLOOM SYNDROME; BLM

CAPN3	GDB:119751	CALPAIN, LARGE POLYPEPTIDE L3; CAPN3 MUSCULAR DYSTROPHY, LIMB-GIRDLE, TYPE 2; LGMD2
CDAN1	GDB:9823267	DYSERYTHROPOIETIC ANEMIA, CONGENITAL, TYPE I
CDAN3	GDB:386192	DYSERYTHROPOIETIC ANEMIA, CONGENITAL, TYPE III; CDAN3
CLN6	GDB:4073043	CEROID-LIPOFUSCINOSIS, NEURONAL 6, LATE INFANTILE, VARIANT; CLN6
CMH3	GDB:138299	CARDIOMYOPATHY, FAMILIAL HYPERTROPHIC, 3; CMH3
CYP19	GDB:119830	CYTOCHROME P450, SUBFAMILY XIX; CYP19
CYP1A1	GDB:120604	CYTOCHROME P450, SUBFAMILY I, POLYPEPTIDE 1; CYP1A1
CYP1A2	GDB:118780	CYTOCHROME P450, SUBFAMILY I, POLYPEPTIDE 2; CYP1A2
DYX1	GDB:1391796	DYSLEXIA, SPECIFIC, 1; DYX1
EPB42	GDB:127385	HEREDITARY HEMOLYTIC PROTEIN 4.2, ERYTHROCYTIC; EPB42
ETFA	GDB:119121	GLUTARICACIDURIA IIA; GA IIA
EYCL3	GDB:4590306	EYE COLOR-3; EYCL3
FAH	GDB:119901	TYROSINEMIA, TYPE I

FBN1	GDB:127115	FIBRILLIN-1; FBN1 MARFAN SYNDROME; MFS
FES	GDB:119906	V-FES FELINE SARCOMA VIRAL/V-FPS FUJINAMI AVIAN SARCOMA VIRAL ONCOGENE
HCVS	GDB:119306	CORONAVIRUS 229E SUSCEPTIBILITY; CVS
HEXA	GDB:120040	TAY-SACHS DISEASE; TSD
IVD	GDB:119354	ISOVALERICACIDEMIA; IVA
LCS1	GDB:11500552	CHOLESTASIS-LYMPHEDEMA SYNDROME
LIPC	GDB:119366	LIPASE, HEPATIC; LIPC
MYO5A	GDB:218824	MYOSIN VA; MYO5A
OCA2	GDB:136820	ALBINISM II
OTSC1	GDB:9860473	OTOSCLEROSIS
PWCR	GDB:120325	PRADER-WILLI SYNDROME
RLBP1	GDB:127341	RETINALDEHYDE-BINDING PROTEIN 1,; RLBP1
SLC12A1	GDB:386121	SOLUTE CARRIER FAMILY 12, MEMBER 1; SLC12A1
SPG6	GDB:511201	SPASTIC PARAPLEGIA 6, AUTOSOMAL DOMINANT; SPG6

TPM1	GDB:127875	TROPOMYOSIN 1; TPM1
UBE3A	GDB:228487	ANGELMAN SYNDROME UBIQUITIN-PROTEIN LIGASE E3A; UBE3A
WMS	GDB:5583902	WEILL-MARCHESANI SYNDROME

Table 18: Genes, Locations and Genetic Disorders on Chromosome 16

Gene	**GDB Accession ID**	**OMIM Link**
ABCC6	GDB:9315106	PSEUDOXANTHOMA ELASTICUM, AUTOSOMAL DOMINANT; PXE PSEUDOXANTHOMA ELASTICUM, AUTOSOMAL RECESSIVE; PXE
ALDOA	GDB:118993	ALDOLASE A, FRUCTOSE-BISPHOSPHATE; ALDOA
APRT	GDB:119003	ADENINE PHOSPHORIBOSYLTRANSFERASE; APRT
ATP2A1	GDB:119716	ATPase, Ca(2+)-TRANSPORTING, FAST-TWITCH 1; ATP2A1 BRODY MYOPATHY
BBS2	GDB:229992	BARDET-BIEDL SYNDROME, TYPE 2; BBS2
CARD15	GDB:11026232	SYNOVITIS, GRANULOMATOUS, WITH UVEITIS AND CRANIAL NEUROPATHIES REGIONAL ENTERITIS
CATM	GDB:701219	MICROPHTHALMIA-CATARACT
CDH1	GDB:120484	CADHERIN 1; CDH1

CETP	GDB:119773	CHOLESTERYL ESTER TRANSFER PROTEIN, PLASMA; CETP
CHST6	GDB:131407	CORNEAL DYSTROPHY, MACULAR TYPE
CLN3	GDB:120593	CEROID-LIPOFUSCINOSIS, NEURONAL 3, JUVENILE; CLN3
CREBBP	GDB:437159	RUBINSTEIN SYNDROME CREB-BINDING PROTEIN; CREBBP
CTH	GDB:119086	CYSTATHIONINURIA
CTM	GDB:119819	CATARACT, ZONULAR
CYBA	GDB:125238	GRANULOMATOUS DISEASE, CHRONIC, AUTOSOMAL CYTOCHROME-b-NEGATIVE FORM
CYLD	GDB:701216	EPITHELIOMA, HEREDITARY MULTIPLE BENIGN CYSTIC
DHS	GDB:9958268	XEROCYTOSIS, HEREDITARY
DNASE1	GDB:132846	DEOXYRIBONUCLEASE I; DNASE1
DPEP1	GDB:128059	RENAL DIPEPTIDASE
ERCC4	GDB:119113	EXCISION-REPAIR, COMPLEMENTING DEFECTIVE, IN CHINESE HAMSTER, 4; ERCC4 XERODERMA PIGMENTOSUM, COMPLEMENTATION GROUP F; XPF
FANCA	GDB:701221	FANCONI ANEMIA, COMPLEMENTATION GROUP A; FACA

GALNS	GDB:129085	MUCOPOLYSACCHARIDOSIS TYPE IVA
GAN	GDB:9864885	NEUROPATHY, GIANT AXONAL; GAN
HAGH	GDB:119292	HYDROXYACYL GLUTATHIONE HYDROLASE; HAGH
HBA1	GDB:119293	HEMOGLOBIN--ALPHA LOCUS-1; HBA1
HBA2	GDB:119294	HEMOGLOBIN--ALPHA LOCUS-2; HBA2
HBHR	GDB:9954541	HEMOGLOBIN H-RELATED MENTAL RETARDATION
HBQ1	GDB:120036	HEMOGLOBIN--THETA-1 LOCUS; HBQ1
HBZ	GDB:119302	HEMOGLOBIN--ZETA LOCUS; HBZ
HBZP	GDB:120037	HEMOGLOBIN--ZETA LOCUS; HBZ
HP	GDB:119314	HAPTOGLOBIN; HP
HSD11B2	GDB:409951	CORTISOL 11-BETA-KETOREDUCTASE DEFICIENCY
IL4R	GDB:118823	INTERLEUKIN-4 RECEPTOR; IL4R
LIPB	GDB:119365	LIPASE B, LYSOSOMAL ACID; LIPB
MC1R	GDB:135162	MELANOCORTIN-1 RECEPTOR; MC1R
MEFV	GDB:125263	MEDITERRANEAN FEVER, FAMILIAL; MEFV

MHC2TA	GDB:6268475	MHC CLASS II TRANSACTIVATOR; MHC2TA
MLYCD	GDB:11500940	MALONYL CoA DECARBOXYLASE DEFICIENCY
PHKB	GDB:120286	PHOSPHORYLASE KINASE, BETA SUBUNIT; PHKB
PHKG2	GDB:140316	PHOSPHORYLASE KINASE, TESTIS/LIVER, GAMMA 2; PHKG2
PKD1	GDB:120293	POLYCYSTIC KIDNEYS POLYCYSTIC KIDNEY DISEASE 1; PKD1
PKDTS	GDB:9954545	POLYCYSTIC KIDNEY DISEASE, INFANTILE SEVERE, WITH TUBEROUS SCLEROSIS;
PMM2	GDB:438697	CARBOHYDRATE-DEFICIENT GLYCOPROTEIN SYNDROME, TYPE I; CDG1 PHOSPHOMANNOMUTASE 2; PMM2
PXE	GDB:6053895	PSEUDOXANTHOMA ELASTICUM, AUTOSOMAL DOMINANT; PXE PSEUDOXANTHOMA ELASTICUM, AUTOSOMAL RECESSIVE; PXE
SALL1	GDB:4216161	TOWNES-BROCKS SYNDROME; TBS SAL-LIKE 1; SALL1
SCA4	GDB:250364	SPINOCEREBELLAR ATAXIA 4; SCA4
SCNN1B	GDB:434471	SODIUM CHANNEL, NONVOLTAGE-GATED 1 BETA; SCNN1B
SCNN1G	GDB:568759	SODIUM CHANNEL, NONVOLTAGE-GATED 1 GAMMA; SCNN1G

TAT	GDB:120398	TYROSINE TRANSAMINASE DEFICIENCY
TSC2	GDB:120466	TUBEROUS SCLEROSIS-2; TSC2
VDI	GDB:119629	DEFECTIVE INTERFERING PARTICLE INDUCTION, CONTROL OF
WT3	GDB:9958957	WILMS TUMOR, TYPE III; WT3

Table 19: Genes, Locations and Genetic Disorders on Chromosome 17

Gene	**GDB Accession ID**	**OMIM Link**
ABR	GDB:119642	ACTIVE BCR-RELATED GENE; ABR
ACACA	GDB:120534	ACETYL-CoA CARBOXYLASE DEFICIENCY
ACADVL	GDB:1248185	ACYL-CoA DEHYDROGENASE, VERY-LONG-CHAIN, DEFICIENCY OF
ACE	GDB:119840	DIPEPTIDYL CARBOXYPEPTIDASE-1; DCP1
ALDH3A2	GDB:1316855	SJOGREN-LARSSON SYNDROME; SLS
APOH	GDB:118887	APOLIPOPROTEIN H; APOH
ASPA	GDB:231014	SPONGY DEGENERATION OF CENTRAL NERVOUS SYSTEM
AXIN2	GDB:9864782	CANCER OF COLON
BCL5	GDB:125178	LEUKEMIA/LYMPHOMA, CHRONIC B-CELL, 5; BCL5

BHD	GDB:11498904	WITH TRICHODISCOMAS AND ACROCHORDONS
BLMH	GDB:3801467	BLEOMYCIN HYDROLASE
BRCA1	GDB:126611	BREAST CANCER, TYPE 1; BRCA1
CACD	GDB:5885801	CHOROIDAL DYSTROPHY, CENTRAL AREOLAR; CACD
CCA1	GDB:118763	CATARACT, CONGENITAL, CERULEAN TYPE 1; CCA1
CCZS	GDB:681973	CATARACT, CONGENITAL ZONULAR, WITH SUTURAL OPACITIES; CCZS
CHRNB1	GDB:120587	CHOLINERGIC RECEPTOR, NICOTINIC, BETA POLYPEPTIDE 1; CHRNB1
CHRNE	GDB:132246	CHOLINERGIC RECEPTOR, NICOTINIC, EPSILON POLYPEPTIDE; CHRNE
CMT1A	GDB:119785	CHARCOT-MARIE-TOOTH DISEASE, TYPE 1A; CMT1A NEUROPATHY, HEREDITARY, WITH LIABILITY TO PRESSURE PALSIES; HNPP
COL1A1	GDB:119061	COLLAGEN, TYPE I, ALPHA-1 CHAIN; COL1A1 OSTEOGENESIS IMPERFECTA TYPE I OSTEOGENESIS IMPERFECTA TYPE IV; OI4
CORD5	GDB:568473	CONE-ROD DYSTROPHY-5; CORD5
CTNS	GDB:700761	CYSTINOSIS, EARLY-ONSET OR INFANTILE NEPHROPATHIC TYPE

EPX	GDB:377700	EOSINOPHIL PEROXIDASE; EPX
ERBB2	GDB:120613	V-ERB-B2 AVIAN ERYTHROBLASTIC LEUKEMIA VIRAL ONCOGENE HOMOLOG 2; ERBB2
G6PC	GDB:231927	GLYCOGEN STORAGE DISEASE I; GSD-I
GAA	GDB:119965	GLYCOGEN STORAGE DISEASE II
GALK1	GDB:119246	GALACTOKINASE DEFICIENCY
GCGR	GDB:304516	GLUCAGON RECEPTOR; GCGR
GFAP	GDB:118799	GLIAL FIBRILLARY ACIDIC PROTEIN; GFAP ALEXANDER DISEASE
GH1	GDB:119982	GROWTH HORMONE 1; GH1
GH2	GDB:119983	GROWTH HORMONE 2; GH2
GP1BA	GDB:118806	GIANT PLATELET SYNDROME
GPSC	GDB:9954564	FAMILIAL PROGRESSIVE SUBCORTICAL
GUCY2D	GDB:136012	AMAUROSIS CONGENITA OF LEBER I GUANYLATE CYCLASE 2D, MEMBRANE; GUC2D CONE-ROD DYSTROPHY-6; CORD6
ITGA2B	GDB:120012	THROMBASTHENIA OF GLANZMANN AND NAEGELI
ITGB3	GDB:120013	INTEGRIN, BETA-3; ITGB3
ITGB4	GDB:128028	INTEGRIN, BETA-4; ITGB4

KRT10	GDB:118828	KERATIN 10; KRT10
KRT12	GDB:5583953	CORNEAL DYSTROPHY, JUVENILE EPITHELIAL, OF MEESMANN KERATIN 12; KRT12
KRT13	GDB:120740	KERATIN 13; KRT13
KRT14	GDB:132145	KERATIN 14; KRT14 GLUTATHIONE SYNTHETASE; GSS
KRT14L1	GDB:120121	KERATIN 14; KRT14
KRT14L2	GDB:120122	KERATIN 14; KRT14
KRT14L3	GDB:120123	KERATIN 14; KRT14
KRT16	GDB:136207	KERATIN 16; KRT16
KRT16L1	GDB:120125	KERATIN 16; KRT16
KRT16L2	GDB:120126	KERATIN 16; KRT16
KRT17	GDB:136211	KERATIN 17; KRT17 PACHYONYCHIA CONGENITA, JACKSON-LAWLER TYPE
KRT9	GDB:303970	HYPERKERATOSIS, LOCALIZED EPIDERMOLYTIC
MAPT	GDB:119434	MICROTUBULE-ASSOCIATED PROTEIN TAU; MAPT PALLIDOPONTONIGRAL DEGENERATION; PPND DISINHIBITION-DEMENTIA-PARKINSONISM-AMYOTROPHY COMPLEX; DDPAC
MDB	GDB:9958959	MEDULLOBLASTOMA; MDB

MDCR	GDB:120525	MILLER-DIEKER LISSENCEPHALY SYNDROME; MDLS PLATELET-ACTIVATING FACTOR ACETYLHYDROLASE, GAMMA SUBUNIT
MGI	GDB:9954550	MYASTHENIA GRAVIS, FAMILIAL INFANTILE; FIMG
MHS2	GDB:132580	MALIGNANT HYPERTHERMIA SUSCEPTIBILITY-2; MHS2
MKS1	GDB:681967	MECKEL SYNDROME; MKS
MPO	GDB:120192	MYELOPEROXIDASE DEFICIENCY
MUL	GDB:636050	MULIBREY NANISM; MUL
MYO15A	GDB:9838006	DEAFNESS, NEUROSENSORY, AUTOSOMAL RECESSIVE, 3; DFNB3
NAGLU	GDB:636533	MUCOPOLYSACCHARIDOSIS TYPE IIIB
NAPB	GDB:9954572	NEURITIS WITH BRACHIAL PREDILECTION; NAPB
NF1	GDB:120231	NEUROFIBROMATOSIS, TYPE I; NF1
NME1	GDB:127965	NON-METASTATIC CELLS 1, PROTEIN EXPRESSED IN; NME1
P4HB	GDB:120708	PROLYL-4-HYDROXYLASE, BETA POLYPEPTIDE; PHDB; PROHB
PAFAH1B1	GDB:677430	MILLER-DIEKER LISSENCEPHALY SYNDROME; MDLS PLATELET-ACTIVATING FACTOR ACETYLHYDROLASE, GAMMA SUBUNIT

PECAM1	GDB:696372	PLATELET-ENDOTHELIAL CELL ADHESION MOLECULE; PECAM1
PEX12	GDB:6155804	ZELLWEGER SYNDROME; ZS PEROXIN-12; PEX12
PHB	GDB:126600	PROHIBITIN; PHB
PMP22	GDB:134190	CHARCOT-MARIE-TOOTH DISEASE, TYPE 1A; CMT1A HYPERTROPHIC NEUROPATHY OF DEJERINE-SOTTAS PERIPHERAL MYELIN PROTEIN 22; PMP22
PRKAR1A	GDB:120313	MYXOMA, SPOTTY PIGMENTATION, AND ENDOCRINE OVERACTIVITY PROTEIN KINASE, cAMP-DEPENDENT, REGULATORY, TYPE I, ALPHA; PRKAR1A
PRKCA	GDB:128015	PROTEIN KINASE C, ALPHA; PRKCA
PRKWNK4	GDB:9954566	PSEUDOHYPOALDOSTERONISM TYPE II, LOCUS B; PHA2B
PRP8	GDB:9957697	RETINITIS PIGMENTOSA-13; RP13
PRPF8	GDB:392647	RETINITIS PIGMENTOSA-13; RP13
PTLAH	GDB:9957342	APLASIA OR HYPOPLASIA
RARA	GDB:120337	RETINOIC ACID RECEPTOR, ALPHA; RARA
RCV1	GDB:135477	RECOVERIN; RCV1
RMSA1	GDB:304519	REGULATOR OF MITOTIC SPINDLE ASSEMBLY 1; RMSA1

RP17	GDB:683199	RETINITIS PIGMENTOSA-17; RP17
RSS	GDB:439249	RUSSELL-SILVER SYNDROME; RSS
SCN4A	GDB:125181	PERIODIC PARALYSIS II
SERPINF2	GDB:120301	PLASMIN INHIBITOR DEFICIENCY
SGCA	GDB:384077	ADHALIN; ADL
SGSH	GDB:1319101	MUCOPOLYSACCHARIDOSIS TYPE IIIA
SHBG	GDB:125280	SEX HORMONE BINDING GLOBULIN; SHBG
SLC2A4	GDB:119997	SOLUTE CARRIER FAMILY 2, MEMBER 4; SLC2A4
SLC4A1	GDB:119874	SOLUTE CARRIER FAMILY 4, ANION EXCHANGER, MEMBER 1; SLC4A1 BLOOD GROUP--DIEGO SYSTEM; DI BLOOD GROUP--WRIGHT ANTIGEN; Wr ELLIPTOCYTOSIS, RHESUS-UNLINKED TYPE HEREDITARY HEMOLYTIC
SLC6A4	GDB:134713	SOLUTE CARRIER FAMILY 6, MEMBER 4; SLC6A4
SMCR	GDB:120379	SMITH-MAGENIS SYNDROME; SMS
SOST	GDB:10450629	SCLEROSTEOSIS
SOX9	GDB:134730	DYSPLASIA
SSTR2	GDB:134186	SOMATOSTATIN RECEPTOR-2; SSTR2

SYM1	GDB:512174	SYMPHALANGISM, PROXIMAL; SYM1
SYNS1	GDB:9862343	SYNOSTOSES, MULTIPLE, WITH BRACHYDACTYLY
TCF2	GDB:125298	TRANSCRIPTION FACTOR-2, HEPATIC; TCF2
THRA	GDB:120730	THYROID HORMONE RECEPTOR, ALPHA 1; THRA
TIMP2	GDB:132612	TISSUE INHIBITOR OF METALLOPROTEINASE-2; TIMP2
TOC	GDB:451978	TYLOSIS WITH ESOPHAGEAL CANCER; TOC
TOP2A	GDB:118884	TOPOISOMERASE (DNA) II, ALPHA; TOP2A
TP53	GDB:120445	CANCER, HEPATOCELLULAR LI-FRAUMENI SYNDROME; LFS TUMOR PROTEIN p53; TP53 CARCINOMA
VBCH	GDB:9954554	HYPEROSTOSIS CORTICALIS GENERALISATA

Table 20: Genes, Locations and Genetic Disorders on Chromosome 18

Gene	**GDB Accession ID**	**OMIM Link**
ATP8B1	GDB:453352	CHOLESTASIS, PROGRESSIVE FAMILIAL INTRAHEPATIC 1; PFIC1 INTRAHEPATIC CHOLESTASIS FAMILIAL INTRAHEPATIC CHOLESTASIS-1; FIC1
BCL2	GDB:119031	B-CELL CLL/LYMPHOMA 2; BCL2

CNSN	GDB:9954580	CARNOSINEMIA
CORD1	GDB:118773	CONE-ROD DYSTROPHY-1; CORD1
CYB5	GDB:125236	METHEMOGLOBINEMIA DUE TO DEFICIENCY OF CYTOCHROME b5
DCC	GDB:119838	DELETED IN COLORECTAL CARCINOMA; DCC
F5F8D	GDB:6919858	FACTOR V AND FACTOR VIII, COMBINED DEFICIENCY OF; F5F8D
FECH	GDB:127282	PROTOPORPHYRIA, ERYTHROPOIETIC
FEO	GDB:4378120	POLYOSTOTIC OSTEOLYTIC DYSPLASIA, HEREDITARY EXPANSILE; HEPOD
LAMA3	GDB:251818	LAMININ, ALPHA 3; LAMA3
LCFS2	GDB:9954578	CANCER
MADH4	GDB:4642788	POLYPOSIS, JUVENILE INTESTINAL MOTHERS AGAINST DECAPENTAPLEGIC, DROSOPHILA, HOMOLOG OF, 4; MADH4
MAFD1	GDB:120163	MANIC-DEPRESSIVE PSYCHOSIS, AUTOSOMAL
MC2R	GDB:135163	ADRENAL UNRESPONSIVENESS TO ACTH
MCL	GDB:9954574	LEIOMYOMATA, HEREDITARY MULTIPLE, OF SKIN
MYP2	GDB:9862232	MYOPIA

NPC1	GDB:138178	NIEMANN-PICK DISEASE, TYPE C1; NPC1
SPPK	GDB:606444	PALMOPLANTARIS STRIATA
TGFBRE	GDB:250852	TRANSFORMING GROWTH FACTOR, BETA 1 RESPONSE ELEMENT
TGIF	GDB:9787150	HOLOPROSENCEPHALY, TYPE 4; HPE4
TTR	GDB:119471	TRANSTHYRETIN; TTR

Table 21: Genes, Locations and Genetic Disorders on Chromosome 19

Gene	**GDB Accession ID**	**OMIM Link**
AD2	GDB:118748	ALZHEIMER DISEASE-2; AD2
AMH	GDB:118996	PERSISTENT MULLERIAN DUCT SYNDROME, TYPES I AND II; PMDS ANTI-MULLERIAN HORMONE; AMH
APOC2	GDB:119689	APOLIPOPROTEIN C-II DEFICIENCY, TYPE I HYPERLIPOPROTEINEMIA DUE TO
APOE	GDB:119691	APOLIPOPROTEIN E; APOE
ATHS	GDB:128803	LIPOPROTEIN PHENOTYPE; ALP
BAX	GDB:228082	BCL2-ASSOCIATED X PROTEIN; BAX
BCKDHA	GDB:119723	MAPLE SYRUP URINE DISEASE
BCL3	GDB:120561	B-CELL LEUKEMIA/LYMPHOMA-3; BCL3

BFIC	GDB:9954584	BENIGN FAMILIAL INFANTILE CONVULSIONS
C3	GDB:119044	COMPLEMENT COMPONENT-3; C3
CACNA1A	GDB:126432	ATAXIA, PERIODIC VESTIBULOCEREBELLAR HEMIPLEGIC MIGRAINE, FAMILIAL; MHP SPINOCEREBELLAR ATAXIA 6; SCA6 CALCIUM CHANNEL, VOLTAGE-DEPENDENT, P/Q TYPE, ALPHA 1A SUBUNIT; CACNA1A
CCO	GDB:119755	CENTRAL CORE DISEASE OF MUSCLE
CEACAM5	GDB:119054	CARCINOEMBRYONIC ANTIGEN; CEA
COMP	GDB:344263	EPIPHYSEAL DYSPLASIA, MULTIPLE; MED PSEUDOACHONDROPLASTIC DYSPLASIA CARTILAGE OLIGOMERIC MATRIX PROTEIN; COMP
CRX	GDB:333932	CONE-ROD DYSTROPHY-2; CORD2 AMAUROSIS CONGENITA OF LEBER I CONE-ROD HOMEO BOX-CONTAINING GENE
DBA	GDB:9600353	ANEMIA, CONGENITAL HYPOPLASTIC, OF BLACKFAN AND DIAMOND
DDU	GDB:10796026	URTICARIA; DDU
DFNA4	GDB:606540	DEAFNESS, AUTOSOMAL DOMINANT NONSYNDROMIC SENSORINEURAL, 4; DFNA4
DLL3	GDB:9959026	VERTEBRAL ANOMALIES

DMPK	GDB:119097	DYSTROPHIA MYOTONICA; DM
DMWD	GDB:7178354	DYSTROPHIA MYOTONICA; DM
DPD1	GDB:10796170	ENGELMANN DISEASE
E11S	GDB:119101	ECHO 11 SENSITIVITY; E11S
ELA2	GDB:118792	ELASTASE-2; ELA2 NEUTROPENIA, CYCLIC
EPOR	GDB:125242	ERYTHROPOIETIN RECEPTOR; EPOR
ERCC2	GDB:119112	EXCISION-REPAIR, COMPLEMENTING DEFECTIVE, IN CHINESE HAMSTER, 2; ERCC2 XERODERMA PIGMENTOSUM IV; XP4
ETFB	GDB:119887	ELECTRON TRANSFER FLAVOPROTEIN, BETA POLYPEPTIDE; ETFB
EXT3	GDB:383780	EXOSTOSES, MULTIPLE, TYPE III; EXT3
EYCL1	GDB:119269	EYE COLOR-1; EYCL1
FTL	GDB:119234	FERRITIN LIGHT CHAIN; FTL
FUT1	GDB:120618	FUCOSYLTRANSFERASE-1; FUT1
FUT2	GDB:120619	FUCOSYLTRANSFERASE-2; FUT2
FUT6	GDB:135180	FUCOSYLTRANSFERASE-6; FUT6
GAMT	GDB:1313736	GUANIDINOACETATE

		METHYLTRANSFERASE; GAMT
GCDH	GDB:136004	GLUTARICACIDEMIA I
GPI	GDB:120015	GLUCOSEPHOSPHATE ISOMERASE; GPI
GUSM	GDB:119291	GLUCURONIDASE, MOUSE, MODIFIER OF; GUSM
HB1	GDB:9954586	BUNDLE BRANCH BLOCK
HCL1	GDB:119304	HAIR COLOR-1; HCL1
HHC2	GDB:249836	HYPOCALCIURIC HYPERCALCEMIA, FAMILIAL, TYPE II; HHC2
HHC3	GDB:9955121	HYPOCALCIURIC HYPERCALCEMIA, FAMILIAL, TYPE III; HHC3
ICAM3	GDB:136236	INTERCELLULAR ADHESION MOLECULE-3; ICAM3
INSR	GDB:119352	INSULIN RECEPTOR; INSR
JAK3	GDB:376460	JANUS KINASE 3 JAK3
KLK3	GDB:119695	ANTIGEN, PROSTATE-SPECIFIC; APS
LDLR	GDB:119362	HYPERCHOLESTEROLEMIA, FAMILIAL; FHC
LHB	GDB:119364	LUTEINIZING HORMONE, BETA POLYPEPTIDE; LHB

LIG1	GDB:127274	LIGASE I, DNA, ATP-DEPENDENT; LIG1
LOH19CR1	GDB:9837482	ANEMIA, CONGENITAL HYPOPLASTIC, OF BLACKFAN AND DIAMOND
LYL1	GDB:120158	LEUKEMIA, LYMPHOID, 1; LYL1
MAN2B1	GDB:119376	MANNOSIDOSIS, ALPHA B, LYSOSOMAL
MCOLN1	GDB:10013974	MUCOLIPIDOSIS IV
MDRV	GDB:6306714	MUSCULAR DYSTROPHY, AUTOSOMAL DOMINANT, WITH RIMMED VACUOLES; MDRV
MLLT1	GDB:136791	MYELOID/LYMPHOID OR MIXED LINEAGE LEUKEMIA, TRANSLOCATED TO, 1; MLLT1
NOTCH3	GDB:361163	DEMENTIA, HEREDITARY MULTI-INFARCT TYPE NOTCH, DROSOPHILA, HOMOLOG OF, 3; NOTCH3
NPHS1	GDB:342105	NEPHROSIS 1, CONGENITAL, FINNISH TYPE; NPHS1
OFC3	GDB:128060	OROFACIAL CLEFT-3; OFC3
OPA3	GDB:9954590	OPTIC ATROPHY, INFANTILE, WITH CHOREA AND SPASTIC PARAPLEGIA
PEPD	GDB:120273	PEPTIDASE D; PEPD
PRPF31	GDB:333911	RETINITIS PIGMENTOSA 11; RP11

PRTN3	GDB:126876	PROTEINASE 3; PRTN3; PR3
PRX	GDB:11501256	HYPERTROPHIC NEUROPATHY OF DEJERINE-SOTTAS
PSG1	GDB:120321	PREGNANCY-SPECIFIC BETA-1-GLYCOPROTEIN 1; PSG1
PVR	GDB:120324	POLIOVIRUS SUSCEPTIBILITY, OR SENSITIVITY; PVS
RYR1	GDB:120359	CENTRAL CORE DISEASE OF MUSCLE HYPERTHERMIA OF ANESTHESIA RYANODINE RECEPTOR-1; RYR1
SLC5A5	GDB:5892184	SOLUTE CARRIER FAMILY 5, MEMBER 5; SLC5A5
SLC7A9	GDB:9958852	CYSTINURIA, TYPE III; CSNU3
STK11	GDB:9732383	PEUTZ-JEGHERS SYNDROME SERINE/THREONINE PROTEIN KINASE 11; STK11
TBXA2R	GDB:127517	THROMBOXANE A2 RECEPTOR, PLATELET; TBXA2R
TGFB1	GDB:120729	ENGELMANN DISEASE TRANSFORMING GROWTH FACTOR, BETA-1; TGFB1
TNNI3	GDB:125309	TROPONIN I, CARDIAC; TNNI3
TYROBP	GDB:9954457	POLYCYSTIC LIPOMEMBRANOUS OSTEODYSPLASIA WITH SCLEROSING LEUKOENCEPHALOPATHY

Table 22: Genes, Locations and Genetic Disorders on Chromosome 20

Gene	GDB Accession ID	OMIM Link
ADA	GDB:119649	ADENOSINE DEAMINASE; ADA
AHCY	GDB:118983	S-ADENOSYLHOMOCYSTEINE HYDROLASE; AHCY
AVP	GDB:119009	DIABETES INSIPIDUS, NEUROHYPOPHYSEAL TYPE ARGININE VASOPRESSIN; AVP
CDAN2	GDB:9823270	DYSERYTHROPOIETIC ANEMIA, CONGENITAL, TYPE II
CDMP1	GDB:438940	CHONDRODYSPLASIA, GREBE TYPE CARTILAGE-DERIVED MORPHOGENETIC PROTEIN 1
CHED1	GDB:3837719	CORNEAL DYSTROPHY, CONGENITAL ENDOTHELIAL; CHED
CHRNA4	GDB:128169	CHOLINERGIC RECEPTOR, NEURONAL NICOTINIC, ALPHA POLYPEPTIDE 4; CHRNA4 EPILEPSY, BENIGN NEONATAL; EBN1
CST3	GDB:119817	AMYLOIDOSIS VI
EDN3	GDB:119862	ENDOTHELIN-3; EDN3 WAARDENBURG-SHAH SYNDROME
EEGV1	GDB:127525	ELECTROENCEPHALOGRAM, LOW-VOLTAGE
FTLL1	GDB:119235	FERRITIN LIGHT CHAIN; FTL
GNAS	GDB:120628	GUANINE NUCLEOTIDE-BINDING

		PROTEIN, ALPHA-STIMULATING POLYPEPTIDE;
GSS	GDB:637022	GLUTATHIONE SYNTHETASE DEFICIENCY OF ERYTHROCYTES, HEMOLYTIC ANEMIA PYROGLUTAMICACIDURIA HNF4AGDB:393281DIABETES MELLITUS, AUTOSOMAL DOMINANT TRANSCRIPTION FACTOR 14, HEPATIC NUCLEAR FACTOR; TCF14
JAG1	GDB:6175920	CHOLESTASIS WITH PERIPHERAL PULMONARY STENOSIS JAGGED 1; JAG1
KCNQ2	GDB:9787229	EPILEPSY, BENIGN NEONATAL; EBN1 POTASSIUM CHANNEL, VOLTAGE-GATED, SUBFAMILY Q, MEMBER 2
MKKS	GDB:9860197	HYDROMETROCOLPOS SYNDROME
NBIA1	GDB:4252819	HALLERVORDEN-SPATZ DISEASE
PCK1	GDB:125349	PHOSPHOENOLPYRUVATE CARBOXYKINASE 1, SOLUBLE; PCK1
PI3	GDB:203940	PROTEINASE INHIBITOR 3; PI3
PPGB	GDB:119507	NEURAMINIDASE DEFICIENCY WITH BETA-GALACTOSIDASE DEFICIENCY
PPMD	GDB:702144	CORNEAL DYSTROPHY, HEREDITARY POLYMORPHOUS POSTERIOR; PPCD
PRNP	GDB:120720	GERSTMANN-STRAUSSLER DISEASE; GSD PRION PROTEIN; PRNP
THBD	GDB:119613	THROMBOMODULIN; THBD

TOP1	GDB:120444	TOPOISOMERASE (DNA) I; TOP1

Table 23: Genes, Locations and Genetic Disorders on Chromosome 21

Gene	GDB Accession ID	OMIM Link
AIRE	GDB:567198	AUTOIMMUNE POLYENDOCRINOPATHY-CANDIDIASIS-ECTODERMAL DYSTROPHY; APECED
APP	GDB:119692	ALZHEIMER DISEASE; AD AMYLOID BETA A4 PRECURSOR PROTEIN; APP
CBS	GDB:119754	HOMOCYSTINURIA
COL6A1	GDB:119065	COLLAGEN, TYPE VI, ALPHA-1 CHAIN; COL6A1 MYOPATHY, BENIGN CONGENITAL, WITH CONTRACTURES
COL6A2	GDB:119793	COLLAGEN, TYPE VI, ALPHA-2 CHAIN; COL6A2 MYOPATHY, BENIGN CONGENITAL, WITH CONTRACTURES
CSTB	GDB:5215249	MYOCLONUS EPILEPSY OF UNVERRICHT AND LUNDBORG CYSTATIN B; CSTB
DCR	GDB:125354	TRISOMY 21
DSCR1	GDB:731000	TRISOMY 21
FPDMM	GDB:9954610	CORE-BINDING FACTOR, RUNT DOMAIN, ALPHA SUBUNIT 2; CBFA2 PLATELET DISORDER, FAMILIAL, WITH ASSOCIATED MYELOID MALIGNANCY

HLCS	GDB:392648	MULTIPLE CARBOXYLASE DEFICIENCY, BIOTIN-RESPONSIVE; MCD
HPE1	GDB:136065	HOLOPROSENCEPHALY, FAMILIAL ALOBAR
ITGB2	GDB:120574	INTEGRIN BETA-2; ITGB2
KCNE1	GDB:127909	POTASSIUM VOLTAGE-GATED CHANNEL, ISK-RELATED SUBFAMILY, MEMBER 1;
KNO	GDB:4073044	KNOBLOCH SYNDROME; KNO
PRSS7	GDB:384083	ENTEROKINASE DEFICIENCY
RUNX1	GDB:128313	CORE-BINDING FACTOR, RUNT DOMAIN, ALPHA SUBUNIT 2; CBFA2 PLATELET DISORDER, FAMILIAL, WITH ASSOCIATED MYELOID MALIGNANCY
SOD1	GDB:119596	AMYOTROPHIC LATERAL SCLEROSIS SUPEROXIDE DISMUTASE-1; SOD1 MUSCULAR ATROPHY, PROGRESSIVE, WITH AMYOTROPHIC LATERAL SCLEROSIS
TAM	GDB:9958709	MYELOPROLIFERATIVE SYNDROME, TRANSIENT

Table 24: Genes, Locations and Genetic Disorders on Chromosome 22

Gene	**GDB Accession ID**	**OMIM Link**
ADSL	GDB:119655	ADENYLOSUCCINATE LYASE; ADSL

ARSA	GDB:119007	METACHROMATIC LEUKODYSTROPHY, LATE-INFANTILE
BCR	GDB:120562	BREAKPOINT CLUSTER REGION; BCR
CECR	GDB:119772	CAT EYE SYNDROME; CES
CHEK2	GDB:9958730	LI-FRAUMENI SYNDROME; LFS OSTEOGENIC SARCOMA
COMT	GDB:119795	CATECHOL-O-METHYLTRANSFERASE; COMT
CRYBB2	GDB:119075	CRYSTALLIN, BETA B2; CRYBB2 CATARACT, CONGENITAL, CERULEAN TYPE, 2; CCA2
CSF2RB	GDB:126838	GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR RECEPTOR, BETA SUBUNIT;
CTHM	GDB:439247	HEART MALFORMATIONS; CTHM
CYP2D6	GDB:132127	CYTOCHROME P450, SUBFAMILY IID; CYP2D
CYP2D@	GDB:119832	CYTOCHROME P450, SUBFAMILY IID; CYP2D
DGCR	GDB:119843	DIGEORGE SYNDROME; DGS
DIA1	GDB:119848	METHEMOGLOBINEMIA DUE TO DEFICIENCY OF METHEMOGLOBIN REDUCTASE
EWSR1	GDB:135984	EWING SARCOMA; EWS

GGT1	GDB:120623	GLUTATHIONURIA
MGCR	GDB:120180	MENINGIOMA; MGM
MN1	GDB:580528	MENINGIOMA; MGM
NAGA	GDB:119445	ALPHA-GALACTOSIDASE B; GALB
NF2	GDB:120232	NEUROFIBROMATOSIS, TYPE II; NF2
OGS2	GDB:9954619	HYPERTELORISM WITH ESOPHAGEAL ABNORMALITY AND HYPOSPADIAS
PDGFB	GDB:120709	V-SIS PLATELET-DERIVED GROWTH FACTOR BETA POLYPEPTIDE; PDGFB
PPARA	GDB:202877	PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR, ALPHA; PPARA
PRODH	GDB:5215168	HYPERPROLINEMIA, TYPE I
SCO2	GDB:9958568	CYTOCHROME c OXIDASE DEFICIENCY
SCZD4	GDB:1387047	SCHIZOPHRENIA DISORDER-4; SCZD4
SERPIND1	GDB:120038	HEPARIN COFACTOR II; HCF2
SLC5A1	GDB:120375	SOLUTE CARRIER FAMILY 5, MEMBER 1; SLC5A1
SOX10	GDB:9834028	SRY-BOX 10; SOX10
TCN2	GDB:119608	TRANSCOBALAMIN II DEFICIENCY

TIMP3	GDB:138175	TISSUE INHIBITOR OF METALLOPROTEINASE-3; TIMP3
VCF	GDB:136422	VELOCARDIOFACIAL SYNDROME

Table 25: Genes, Locations and Genetic Disorders on Chromosome X

Gene	**GDB Accession ID**	**OMIM Link**
ABCD1	GDB:118991	ADRENOLEUKODYSTROPHY; ALD
ACTL1	GDB:119648	ACTIN-LIKE SEQUENCE-1; ACTL1
ADFN	GDB:118977	ALBINISM-DEAFNESS SYNDROME; ADFN; ALDS
AGMX2	GDB:119661	AGAMMAGLOBULINEMIA, X-LINKED, TYPE 2; AGMX2; XLA2
AHDS	GDB:125899	MENTAL RETARDATION, X-LINKED, WITH HYPOTONIA
AIC	GDB:118986	CORPUS CALLOSUM, AGENESIS OF, WITH CHORIORETINAL ABNORMALITY
AIED	GDB:119663	ALBINISM, OCULAR, TYPE 2; OA2
AIH3	GDB:131443	AMELOGENESIS IMPERFECTA-3, HYPOPLASTIC TYPE; AIH3
ALAS2	GDB:119666	ANEMIA, HYPOCHROMIC
AMCD	GDB:5584286	ARTHROGRYPOSIS MULTIPLEX CONGENITA, DISTAL

AMELX	GDB:119675	AMELOGENESIS IMPERFECTA-1, HYPOPLASTIC TYPE; AIH1
ANOP1	GDB:128454	CLINICAL; ANOP1
AR	GDB:120556	ANDROGEN INSENSITIVITY SYNDROME; AIS ANDROGEN RECEPTOR; AR
ARAF1	GDB:119004	V-RAF MURINE SARCOMA 3611 VIRAL ONCOGENE HOMOLOG 1; ARAF1
ARSC2	GDB:119702	ARYLSULFATASE C, f FORM; ARSC2
ARSE	GDB:555743	CHONDRODYSPLASIA PUNCTATA 1, X-LINKED RECESSIVE; CDPX1
ARTS	GDB:9954651	FATAL X-LINKED, WITH DEAFNESS AND LOSS OF VISION
ASAT	GDB:9954649	SIDEROBLASTIC, AND SPINOCEREBELLAR ATAXIA; ASAT
ASSP5	GDB:119019	CITRULLINEMIA
ATP7A	GDB:119395	ATPase, Cu(2+)-TRANSPORTING, ALPHA POLYPEPTIDE; ATP7A MENKES SYNDROME
ATRX	GDB:136052	ALPHA-THALASSEMIA/MENTAL RETARDATION SYNDROME, X-LINKED; ATRX ALPHA-THALASSEMIA/MENTAL RETARDATION SYNDROME, NONDELETION TYPE
AVPR2	GDB:131475	DIABETES INSIPIDUS, NEPHROGENIC
BFLS	GDB:120566	BORJESON SYNDROME; BORJ

BGN	GDB:119727	BIGLYCAN; BGN
BTK	GDB:120542	BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE; BTK
BZX	GDB:5205912	BAZEX SYNDROME; BZX
C1HR	GDB:119040	TATA BOX BINDING PROTEIN (TBP)-ASSOCIATED FACTOR 2A; TAF2A
CACNA1F	GDB:6053864	NIGHTBLINDNESS, CONGENITAL STATIONARY, X-LINKED, TYPE 2; CSNB2 CALCIUM CHANNEL, VOLTAGE-DEPENDENT, ALPHA 1F SUBUNIT; CACNA1F
CALB3	GDB:133780	CALBINDIN 3; CALB3
CBBM	GDB:9958963	COLORBLINDNESS, BLUE-MONO-CONE-MONOCHROMATIC TYPE; CBBM
CCT	GDB:119756	CATARACT, CONGENITAL TOTAL, WITH POSTERIOR SUTURAL OPACITIES IN HETEROZYGOTES;
CDR1	GDB:119053	CEREBELLAR DEGENERATION-RELATED AUTOANTIGEN-1; CDR1; CDR34
CFNS	GDB:9579470	CRANIOFRONTONASAL SYNDROME; CFNS
CGF1	GDB:6275867	COGNITION
CHM	GDB:120400	CHOROIDEREMIA; CHM
CHR39C	GDB:119779	CHOLESTEROL REPRESSIBLE PROTEIN 39C; CHR39C

CIDX	GDB:127736	SEVERE COMBINED IMMUNODEFICIENCY DISEASE, X-LINKED, 2; SCIDX2
CLA2	GDB:119782	CEREBELLAR ATAXIA, X-LINKED; CLA2
CLCN5	GDB:270667	CHLORIDE CHANNEL 5; CLCN5 FANCONI SYNDROME, RENAL, WITH NEPHROCALCINOSIS AND RENAL STONES NEPHROLITHIASIS, X-LINKED RECESSIVE, WITH RENAL FAILURE; XRN
CLS	GDB:119784	RIBOSOMAL PROTEIN S6 KINASE, 90 KD, POLYPEPTIDE 3; RPS6KA3 COFFIN-LOWRY SYNDROME; CLS
CMTX2	GDB:128311	CHARCOT-MARIE-TOOTH NEUROPATHY, X-LINKED RECESSIVE, 2; CMTX2
CMTX3	GDB:128151	CHARCOT-MARIE-TOOTH NEUROPATHY, X-LINKED RECESSIVE, 3; CMTX3
CND	GDB:9954627	DERMOIDS OF CORNEA; CND
COD1	GDB:119787	CONE DYSTROPHY, X-LINKED, 1; COD1
COD2	GDB:6520166	CONE DYSTROPHY, X-LINKED, 2; COD2
COL4A5	GDB:120596	COLLAGEN, TYPE IV, ALPHA-5 CHAIN; COL4A5 LEIOMYOMATOSIS, ESOPHAGEAL AND VULVAL, WITH NEPHROPATHY
COL4A6	GDB:222775	COLLAGEN, TYPE IV, ALPHA-6 CHAIN; COL4A6 LEIOMYOMATOSIS, ESOPHAGEAL AND VULVAL, WITH NEPHROPATHY
CPX	GDB:120598	CLEFT PALATE, X-LINKED; CPX

CVD1	GDB:9954659	CARDIAC VALVULAR DYSPLASIA, X-LINKED
CYBB	GDB:120513	GRANULOMATOUS DISEASE, CHRONIC; CGD
DCX	GDB:9823272	LISSENCEPHALY, X-LINKED
DFN2	GDB:119091	DEAFNESS, X-LINKED 2, PERCEPTIVE CONGENITAL; DFN2
DFN4	GDB:433255	DEAFNESS, X-LINKED 4, CONGENITAL SENSORINEURAL; DFN4
DFN6	GDB:1320698	DEAFNESS, X-LINKED, 6, PROGRESSIVE; DFN6
DHOF	GDB:119847	FOCAL DERMAL HYPOPLASIA; DHOF
DIAPH2	GDB:9835484	DIAPHANOUS, DROSOPHILA, HOMOLOG OF, 2 DKC1GDB:119096 DYSKERATOSIS CONGENITA; DKC
DMD	GDB:119850	MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE AND BECKER
DSS	GDB:433750	DOSAGE-SENSITIVE SEX REVERSAL; DSS
DYT3	GDB:118789	TORSION DYSTONIA-3, X-LINKED TYPE; DYT3
EBM	GDB:119102	BULLOUS DYSTROPHY, HEREDITARY MACULAR TYPE
EBP	GDB:125212	CHONDRODYSPLASIA PUNCTATA, X-LINKED DOMINANT; CDPX2; CDPXD;

		CPXD
ED1	GDB:119859	ECTODERMAL DYSPLASIA, ANHIDROTIC; EDA
ELK1	GDB:119867	ELK1, MEMBER OF ETS ONCOGENE FAMILY; ELK1
EMD	GDB:119108	MUSCULAR DYSTROPHY, TARDIVE, DREIFUSS-EMERY TYPE, WITH CONTRACTURES
EVR2	GDB:136068	EXUDATIVE VITREORETINOPATHY, FAMILIAL, X-LINKED RECESSIVE; EVR2
F8C	GDB:119124	HEMOPHILIA A
F9	GDB:119900	HEMOPHILIA B; HEMB
FCP1	GDB:347490	F-CELL PRODUCTION, X-LINKED; FCPX
FDPSL5	GDB:119922	SYNTHETASE-5; FPSL5
FGD1	GDB:119131	SYNDROME FACIOGENITAL DYSPLASIA; FGDY
FGS1	GDB:9836950	FG SYNDROME
FMR1	GDB:129038	FRAGILE SITE MENTAL RETARDATION-1; FMR1
FMR2	GDB:141566	FRAGILE SITE, FOLIC ACID TYPE, RARE, FRA(X)(q28); FRAXE
G6PD	GDB:120621	GLUCOSE-6-PHOSPHATE

		DEHYDROGENASE; G6PD
GABRA3	GDB:119968	GAMMA-AMINOBUTYRIC ACID RECEPTOR, ALPHA-3; GABRA3
GATA1	GDB:125373	GATA-BINDING PROTEIN 1; GATA1
GDI1	GDB:1347097	GDP DISSOCIATION INHIBITOR 1; GDI1 MENTAL RETARDATION, X-LINKED NONSPECIFIC, TYPE 3; MRX3
GDXY	GDB:9954629	DYSGENESIS, XY FEMALE TYPE; GDXY
GJB1	GDB:125246	CHARCOT-MARIE-TOOTH PERONEAL MUSCULAR ATROPHY, X-LINKED; CMTX1 GAP JUNCTION PROTEIN, BETA-1, 32 KD; GJB1
GK	GDB:119271	HYPERGLYCEROLEMIA
GLA	GDB:119272	ANGIOKERATOMA, DIFFUSE
GPC3	GDB:3770726	GLYPICAN-3; GPC3 SIMPSON DYSMORPHIA SYNDROME; SDYS
GRPR	GDB:128035	GASTRIN-RELEASING PEPTIDE RECEPTOR; GRPR
GTD	GDB:9954635	GONADOTROPIN DEFICIENCY; GTD
GUST	GDB:9954655	MENTAL RETARDATION WITH OPTIC ATROPHY, DEAFNESS, AND SEIZURES
HMS1	GDB:251827	1; HMS1

HPRT1	GDB:119317	HYPOXANTHINE GUANINE PHOSPHORIBOSYLTRANSFERASE 1; HPRT1
HPT	GDB:119322	HYPOPARATHYROIDISM, X-LINKED; HYPX
HTC2	GDB:700980	HYPERTRICHOSIS, CONGENITAL GENERALIZED; CGH; HCG
HTR2C	GDB:378202	5-@HYDROXYTRYPTAMINE RECEPTOR 2C; HTR2C
HYR	GDB:9954625	REGULATOR; HYR
IDS	GDB:120521	MUCOPOLYSACCHARIDOSIS TYPE II
IHG1	GDB:119343	HYPOPLASIA OF, WITH GLAUCOMA; IHG
IL2RG	GDB:134807	INTERLEUKIN-2 RECEPTOR, GAMMA; IL2RG SEVERE COMBINED IMMUNODEFICIENCY DISEASE, X-LINKED, 2; SCIDX2
INDX	GDB:9954657	IMMUNONEUROLOGIC DISORDER, X-LINKED
IP1	GDB:120105	INCONTINENTIA PIGMENTI, TYPE I; IP1
IP2	GDB:120106	INCONTINENTIA PIGMENTI, TYPE II; IP2
JMS	GDB:204055	MENTAL RETARDATION, X-LINKED, WITH GROWTH RETARDATION, DEAFNESS, AND
KAL1	GDB:120116	KALLMANN SYNDROME 1; KAL1
KFSD	GDB:128174	KERATOSIS FOLLICULARIS SPINULOSA

		DECALVANS CUM OPHIASI; KFSD
L1CAM	GDB:120133	CLASPED THUMB AND MENTAL RETARDATION L1 CELL ADHESION MOLECULE; L1CAM
LAMP2	GDB:125376	LYSOSOME-ASSOCIATED MEMBRANE PROTEIN B; LAMP2; LAMPB
MAA	GDB:119372	MICROPHTHALMIA OR ANOPHTHALMOS, WITH ASSOCIATED ANOMALIES; MAA
MAFD2	GDB:119373	PSYCHOSIS, X-LINKED
MAOA	GDB:120164	MONOAMINE OXIDASE A; MAOA
MAOB	GDB:119377	MONOAMINE OXIDASE B; MAOB
MCF2	GDB:120168	MCF.2 CELL LINE DERIVED TRANSFORMING SEQUENCE; MCF2
MCS	GDB:128370	MENTAL RETARDATION, X-LINKED, SYNDROMIC-4, WITH CONGENITAL CONTRACTURES
MEAX	GDB:119383	X-LINKED, WITH EXCESSIVE AUTOPHAGY; XMEA; MEAX
MECP2	GDB:3851454	SYNDROME; RTT
MF4	GDB:119386	METACARPAL 4-5 FUSION; MF4
MGC1	GDB:120179	MEGALOCORNEA; MGC1; MGCN
MIC5	GDB:120526	SURFACE ANTIGEN, X-LINKED; SAX

MID1	GDB:9772232	OPITZ SYNDROME
MLLT7	GDB:392309	MYELOID/LYMPHOID OR MIXED-LINEAGE LEUKEMIA, TRANSLOCATED TO, 7; MLLT7
MLS	GDB:262123	MICROPHTHALMIA WITH LINEAR SKIN DEFECTS; MLS
MRSD	GDB:119398	MENTAL RETARDATION, SKELETAL DYSPLASIA, AND ABDUCENS PALSY; MRSD
MRX14	GDB:138453	RETARDATION, X-LINKED 14; MRX14
MRX1	GDB:120193	MENTAL RETARDATION, X-LINKED NONSPECIFIC, TYPE 1; MRX1
MRX20	GDB:217050	MENTAL RETARDATION, X-LINKED 20; MRX20
MRX2	GDB:120194	RETARDATION, X-LINKED NONSPECIFIC, TYPE 2; MRX2
MRX3	GDB:128105	GDP DISSOCIATION INHIBITOR 1; GDI1 MENTAL RETARDATION, X-LINKED NONSPECIFIC, TYPE 3; MRX3
MRX40	GDB:700754	MENTAL RETARDATION, X-LINKED, WITH HYPOTONIA
MRXA	GDB:9954641	MENTAL RETARDATION, X-LINKED NONSPECIFIC, WITH APHASIA; MRXA
MSD	GDB:119399	SYNDROME
MTM1	GDB:119439	MYOTUBULAR MYOPATHY 1; MTM1

MYCL2	GDB:120209	MYCL-RELATED PROCESSED GENE; MYCL2
MYP1	GDB:127783	MYOPIA, X-LINKED; MYP1
NDP	GDB:119449	NORRIE DISEASE; NDP
NHS	GDB:120235	CATARACT-DENTAL SYNDROME
NPHL1	GDB:433705	NEPHROLITHIASIS, X-LINKED RECESSIVE, WITH RENAL FAILURE; XRN
NR0B1	GDB:118982	ADRENAL HYPOPLASIA, CONGENITAL; AHC
NSX	GDB:125596	SYNDROME; NSX
NYS1	GDB:119458	NYSTAGMUS, X-LINKED; NYS
NYX	GDB:119814	NIGHTBLINDNESS, CONGENITAL STATIONARY, WITH MYOPIA; CSNB1
OA1	GDB:119459	ALBINISM, OCULAR, TYPE 1; OA1
OASD	GDB:138457	OCULAR, WITH LATE-ONSET SENSORINEURAL DEAFNESS; OASD
OCRL	GDB:119461	LOWE OCULOCEREBRORENAL SYNDROME; OCRL
ODT1	GDB:125360	TEETH, ABSENCE OF
OFD1	GDB:120248	OROFACIODIGITAL SYNDROME 1; OFD1

OPA2	GDB:125358	OPTIC ATROPHY 2; OPA2
OPD1	GDB:120249	OTOPALATODIGITAL SYNDROME
OPEM	GDB:119467	OPHTHALMOPLEGIA, EXTERNAL, AND MYOPIA; OPEM
OPN1LW	GDB:120724	COLORBLINDNESS, PARTIAL, PROTAN SERIES; CBP
OPN1MW	GDB:120622	COLORBLINDNESS, PARTIAL, DEUTAN SERIES; CBD; DCB
OTC	GDB:119468	ORNITHINE TRANSCARBAMYLASE DEFICIENCY, HYPERAMMONEMIA DUE TO; OTC
P3	GDB:9954667	PROTEIN P3
PDHA1	GDB:118895	PYRUVATE DEHYDROGENASE COMPLEX, E1-ALPHA POLYPEPTIDE-1; PDHA1
PDR	GDB:203409	AMYLOIDOSIS, FAMILIAL CUTANEOUS
PFC	GDB:120275	PROPERDIN DEFICIENCY, X-LINKED
PFKFB1	GDB:125375	6-@PHOSPHOFRUCTO-2-KINASE; PFKFB1
PGK1	GDB:120282	PHOSPHOGLYCERATE KINASE 1; PGK1
PGK1P1	GDB:120283	PHOSPHOGLYCERATE KINASE 1; PGK1
PGS	GDB:128372	DANDY-WALKER MALFORMATION WITH MENTAL RETARDATION, BASAL GANGLIA DISEASE,

PHEX	GDB:120520	HYPOPHOSPHATEMIA, VITAMIN D-RESISTANT RICKETS; HYP
PHKA1	GDB:120285	PHOSPHORYLASE KINASE, ALPHA 1 SUBUNIT (MUSCLE); PHKA1
PHKA2	GDB:127279	GLYCOGEN STORAGE DISEASE VIII
PHP	GDB:119494	PANHYPOPITUITARISM; PHP
PIGA	GDB:138138	PHOSPHATIDYLINOSITOL GLYCAN, CLASS A; PIGA
PLP1	GDB:120302	PROTEOLIPID PROTEIN, MYELIN; PLP
POF1	GDB:120716	PREMATURE OVARIAN FAILURE 1; POF1
POLA	GDB:120304	POLYMERASE, DNA, ALPHA; POLA
POU3F4	GDB:351386	DEAFNESS, CONDUCTIVE, WITH STAPES FIXATION
PPMX	GDB:9954669	RETARDATION WITH PSYCHOSIS, PYRAMIDAL SIGNS, AND MACROORCHIDISM
PRD	GDB:371323	DYSPLASIA, PRIMARY
PRPS1	GDB:120318	PHOSPHORIBOSYLPYROPHOSPHATE SYNTHETASE-I; PRPS1
PRPS2	GDB:120320	PHOSPHORIBOSYLPYROPHOSPHATE SYNTHETASE-II; PRPS2
PRS	GDB:128368	MENTAL RETARDATION, X-LINKED,

		SYNDROMIC-2, WITH DYSMORPHISM AND CEREBRAL
PRTS	GDB:128367	PARTINGTON X-LINKED MENTAL RETARDATION SYNDROME; PRTS
PSF2	GDB:119519	TRANSPORTER 2, ABC; TAP2
RENBP	GDB:133792	RENIN-BINDING PROTEIN; RENBP
RENS1	GDB:9806348	MENTAL RETARDATION, X-LINKED, RENPENNING TYPE
RP2	GDB:120353	RETINITIS PIGMENTOSA-2; RP2
RP6	GDB:125381	PIGMENTOSA-6; RP6
RPGR	GDB:118736	RETINITIS PIGMENTOSA-3; RP3
RPS4X	GDB:128115	RIBOSOMAL PROTEIN S4, X-LINKED; RPS4X
RPS6KA3	GDB:365648	RIBOSOMAL PROTEIN S6 KINASE, 90 KD, POLYPEPTIDE 3; RPS6KA3
RS1	GDB:119581	RETINOSCHISIS; RS
S11	GDB:120361	ANTIGEN, X-LINKED, SECOND; SAX2
SDYS	GDB:119590	GLYPICAN-3; GPC3 SIMPSON DYSMORPHIA SYNDROME; SDYS
SEDL	GDB:120372	SPONDYLOEPIPHYSEAL DYSPLASIA, LATE; SEDL

SERPINA7	GDB:120399	THYROXINE-BINDING GLOBULIN OF SERUM; TBG
SH2D1A	GDB:120701	IMMUNODEFICIENCY, X-LINKED PROGRESSIVE COMBINED VARIABLE
SHFM2	GDB:226635	SPLIT-HAND/SPLIT-FOOT ANOMALY, X-LINKED
SHOX	GDB:6118451	SHORT STATURE; SS
SLC25A5	GDB:125190	ADENINE NUCLEOTIDE TRANSLOCATOR 2; ANT2
SMAX2	GDB:9954643	SPINAL MUSCULAR ATROPHY, X-LINKED LETHAL INFANTILE
SRPX	GDB:3811398	RETINITIS PIGMENTOSA-3; RP3
SRS	GDB:136337	MENTAL RETARDATION, X-LINKED, SNYDER-ROBINSON TYPE
STS	GDB:120393	ICHTHYOSIS, X-LINKED
SYN1	GDB:119606	SYNAPSIN I; SYN1
SYP	GDB:125295	SYNAPTOPHYSIN; SYP
TAF1	GDB:120573	TATA BOX BINDING PROTEIN (TBP)-ASSOCIATED FACTOR 2A; TAF2A
TAZ	GDB:120609	CARDIOMYOPATHY, DILATED 3A; CMD3A ENDOCARDIAL FIBROELASTOSIS-2; EFE2
TBX22	GDB:10796448	CLEFT PALATE, X-LINKED; CPX

TDD	GDB:119610	MALE PSEUDOHERMAPHRODITISM: DEFICIENCY OF TESTICULAR 17,20-DESMOLASE;
TFE3	GDB:125870	TRANSCRIPTION FACTOR FOR IMMUNOGLOBULIN HEAVY-CHAIN ENHANCER-3; TFE3
THAS	GDB:128158	THORACOABDOMINAL SYNDROME; TAS
THC	GDB:125361	THROMBOCYTOPENIA, X-LINKED; THC; XLT
TIMM8A	GDB:119090	DEAFNESS 1, PROGRESSIVE; DFN1
TIMP1	GDB:119615	TISSUE INHIBITOR OF METALLOPROTEINASE-1; TIMP1
TKCR	GDB:119616	TORTICOLLIS, KELOIDS, CRYPTORCHIDISM, AND RENAL DYSPLASIA; TKC
TNFSF5	GDB:120632	IMMUNODEFICIENCY WITH INCREASED IgM
UBE1	GDB:118954	UBIQUITIN-ACTIVATING ENZYME 1; UBE1
UBE2A	GDB:131647	UBIQUITIN-CONJUGATING ENZYME E2A; UBE2A
WAS	GDB:120736	WISKOTT-ALDRICH SYNDROME; WAS
WSN	GDB:125864	PARKINSONISM, EARLY-ONSET, WITH MENTAL RETARDATION
WTS	GDB:128373	MENTAL RETARDATION, X-LINKED, SYNDROMIC-6, WITH GYNECOMASTIA

		AND OBESITY;
WWS	GDB:120497	WIEACKER SYNDROME
XIC	GDB:120498	X-INACTIVATION-SPECIFIC TRANSCRIPT; XIST
XIST	GDB:126428	X-INACTIVATION-SPECIFIC TRANSCRIPT; XIST
XK	GDB:120499	Xk LOCUS
XM	GDB:119634	XM SYSTEM
XS	GDB:119636	LUTHERAN SUPPRESSOR, X-LINKED; XS; LUXS
ZFX	GDB:120502	ZINC FINGER PROTEIN, X-LINKED; ZFX
ZIC3	GDB:249141	HETEROTAXY, X-LINKED VISCERAL; HTX1
ZNF261	GDB:9785766	MENTAL RETARDATION, X-LINKED; DXS6673E
ZNF41	GDB:125865	ZINC FINGER PROTEIN-41; ZNF41
ZNF6	GDB:120508	ZINC FINGER PROTEIN-6; ZNF6

Table 26: Genes, Locations and Genetic Disorders on Chromosome Y

Gene	**GDB Accession ID**	**OMIM Link**
AMELY	GDB:119676	AMELOGENIN, Y-CHROMOSOMAL; AMELY

ASSP6	GDB:119020	CITRULLINEMIA
AZF1	GDB:119027	AZOOSPERMIA FACTOR 1; AZF1
AZF2	GDB:456131	AZOOSPERMIA FACTOR 2; AZF2
DAZ	GDB:635890	DELETED IN AZOOSPERMIA; DAZ
GCY	GDB:119267	CONTROL, Y-CHROMOSOME INFLUENCED; GCY
RPS4Y	GDB:128052	RIBOSOMAL PROTEIN S4, Y-LINKED; RPS4Y
SMCY	GDB:5875390	HISTOCOMPATIBILITY Y ANTIGEN; HY; HYA
SRY	GDB:125556	SEX-DETERMINING REGION Y; SRY
ZFY	GDB:120503	ZINC FINGER PROTEIN, Y-LINKED; ZFY

Table 27: Genes, Locations and Genetic Disorders in Unknown or Multiple Locations

Gene	**GDB Accession ID**	**OMIM Link**
ABAT	GDB:581658	GAMMA-AMINOBUTYRATE TRANSAMINASE
AEZ	GDB:128360	ACRODERMATITIS ENTEROPATHICA, ZINC-DEFICIENCY TYPE; AEZ
AFA	GDB:265277	FILIFORME ADNATUM AND CLEFT PALATE
AFD1	GDB:265292	DYSOSTOSIS, TREACHER COLLINS TYPE, WITHLIMB ANOMALIES

AGS1	GDB:10795417	ENCEPHALOPATHY, FAMILIAL INFANTILE, WITH CALCIFICATION OF BASAL GANGLIA
ASAH	GDB:6837715	FARBER LIPOGRANULOMATOSIS
ASD1	GDB:6276019	ATRIAL SEPTAL DEFECT; ASD
ASMT	GDB:136259	CETYLSEROTONIN METHYLTRANSFERASE; ASMT ACETYLSEROTONIN METHYLTRANSFERASE, Y-CHROMOSOMAL; ASMTY; HIOMTY
BCH	GDB:118758	CHOREA, HEREDITARY BENIGN; BCH
CCAT	GDB:118738	CATARACT, CONGENITAL OR JUVENILE
CECR9	GDB:10796163	CAT EYE SYNDROME; CES
CEPA	GDB:581848	CONTROL, CONGENITAL FAILURE OF
CHED2	GDB:9957389	CORNEAL DYSTROPHY, CONGENITAL HEREDITARY
CLA1	GDB:119781	CEREBELLOPARENCHYMAL DISORDER III
CLA3	GDB:128453	CEREBELLOPARENCHYMAL DISORDER I; CPD I
CLN4	GDB:125229	CEROID-LIPOFUSCINOSIS, NEURONAL 4; CLN4
CPO	GDB:119070	COPROPORPHYRIA
CSF2RA	GDB:118777	COLONY STIMULATING FACTOR 2 RECEPTOR, ALPHA; CSF2RA

		GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR RECEPTOR, ALPHA SUBUNIT,
CTS1	GDB:118779	CARPAL TUNNEL SYNDROME; CTS; CTS1
DF	GDB:132645	FACTOR D
DIH1	GDB:439243	DIAPHRAGMATIC
DWS	GDB:128371	SYNDROME; DWS
DYT2	GDB:118788	DYSTONIA MUSCULORUM DEFORMANS 2; DYT2
DYT4	GDB:433751	DYSTONIA MUSCULORUM DEFORMANS 4; DYT4
EBR3	GDB:118739	EPIDERMOLYSIS BULLOSA DYSTROPHICA NEUROTROPHICA
ECT	GDB:128640	CENTRALOPATHIC EPILEPSY
EEF1A1L14	GDB:1327185	PROSTATIC CARCINOMA ONCOGENE PTI-1
EYCL2	GDB:4642815	EYE COLOR-3; EYCL3
FA1	GDB:118795	FANCONI ANEMIA, COMPLEMENTATION GROUP A; FACA
FANCB	GDB:9864269	FANCONI PANCYTOPENIA, TYPE 2
GCSH	GDB:126842	HYPERGLYCINEMIA, ISOLATED NONKETOTIC, TYPE III; NKH3

GCSL	GDB:132139	ISOLATED NONKETOTIC, TYPE IV; NKH4
GDF5	GDB:433948	CARTILAGE-DERIVED MORPHOGENETIC PROTEIN 1
GIP	GDB:119985	GASTRIC INHIBITORY POLYPEPTIDE; GIP
GTS	GDB:118807	GILLES DE LA TOURETTE SYNDROME; GTS
HHG	GDB:118740	HYPERGONADOTROPIC HYPOGONADISM; HHG
HMI	GDB:265275	OF ITO; HMI
HOAC	GDB:118812	DEAFNESS, CONGENITAL, AUTOSOMAL RECESSIVE
HOKPP2	GDB:595535	HYPOKALEMIC PERIODIC PARALYSIS, TYPE II; HOKPP2
HRPT1	GDB:125252	HYPERPARATHYROIDISM, FAMILIAL PRIMARY
HSD3B3	GDB:676973	GIANT CELL HEPATITIS, NEONATAL
HTC1	GDB:265286	HYPERTRICHOSIS UNIVERSALIS CONGENITA, AMBRAS TYPE; HTC1
HV1S	GDB:9955009	HERPES VIRUS SENSITIVITY; HV1S
ICR1	GDB:127785	LAMELLAR, AUTOSOMAL DOMINANT FORM
ICR5	GDB:127789	ICHTHYOSIS CONGENITA, HARLEQUIN FETUS TYPE

IL3RA	GDB:128985	INTERLEUKIN-3 RECEPTOR, ALPHA; IL3RA INTERLEUKIN-3 RECEPTOR, Y-CHROMOSOMAL; IL3RA
KAL2	GDB:265288	KALLMANN SYNDROME 2; KAL2
KMS	GDB:118827	SYNDROME; KMS
KRT18	GDB:120127	KERATIN 18; KRT18
KSS	GDB:9957718	KEARNS-SAYRE SYNDROME; KSS
LCAT	GDB:119359	FISH-EYE DISEASE; FED LECITHIN:CHOLESTEROL ACYLTRANSFERASE DEFICIENCY
LIMM	GDB:9958161	MYOPATHY, MITOCHONDRIAL, LETHAL INFANTILE; LIMM
MANBB	GDB:125262	MANNOSIDOSIS, BETA; MANB1
MCPH2	GDB:9863035	MICROCEPHALY; MCT
MEB	GDB:599557	DISEASE
MELAS	GDB:9955855	MELAS SYNDROME
MIC2	GDB:120184	SURFACE ANTIGEN MIC2; MIC2; CD99 MIC2 SURFACE ANTIGEN, Y-CHROMOSOMAL; MIC2Y
MPFD	GDB:439372	CONGENITAL, WITH FIBER-TYPE DISPROPORTION
MS	GDB:229116	SCLEROSIS; MS

MSS	GDB:118743	MARINESCO-SJOGREN SYNDROME; MSS
MTATP6	GDB:118897	ATP SYNTHASE 6; MTATP6
MTCO1	GDB:118900	COMPLEX IV, CYTOCHROME c OXIDASE SUBUNIT I; MTCO1; COI
MTCO3	GDB:118902	CYTOCHROME c OXIDASE III; MTCO3
MTCYB	GDB:118906	COMPLEX III, CYTOCHROME b SUBUNIT
MTND1	GDB:118911	COMPLEX I, SUBUNIT ND1; MTND1
MTND2	GDB:118912	COMPLEX I, SUBUNIT ND2; MTND2
MTND4	GDB:118914	COMPLEX I, SUBUNIT ND4; MTND4
MTND5	GDB:118916	COMPLEX I, SUBUNIT ND5; MTND5
MTND6	GDB:118917	COMPLEX I, SUBUNIT ND6; MTND6
MTRNR1	GDB:118920	RIBOSOMAL RNA, MITOCHONDRIAL, 12S; MTRNR1
MTRNR2	GDB:118921	RIBOSOMAL RNA, MITOCHONDRIAL, 16S; MTRNR2
MTTE	GDB:118926	TRANSFER RNA, MITOCHONDRIAL, GLUTAMIC ACID; MTTE
MTTG	GDB:118933	TRANSFER RNA, MITOCHONDRIAL, GLYCINE; MTTG
MTTI	GDB:118935	TRANSFER RNA, MITOCHONDRIAL,

		ISOLEUCINE; MTTI
MTTK	GDB:118936	MERRF SYNDROME TRANSFER RNA, MITOCHONDRIAL, LYSINE; MTTK
MTTL1	GDB:118937	MERRF SYNDROME TRANSFER RNA, MITOCHONDRIAL, LEUCINE, 1; MTTL1
MTTL2	GDB:118938	TRANSFER RNA, MITOCHONDRIAL, LEUCINE, 2; MTTL2
MTTN	GDB:118940	TRANSFER RNA, MITOCHONDRIAL, ASPARAGINE; MTTN
MTTP	GDB:118941	TRANSFER RNA, MITOCHONDRIAL, PROLINE; MTTP
MTTS1	GDB:118944	TRANSFER RNA, MITOCHONDRIAL, SERINE, 1; MTTS1
NAMSD	GDB:681237	NEUROPATHY, MOTOR-SENSORY, TYPE II, WITH DEAFNESS AND MENTAL RETARDATION
NODAL	GDB:9848762	NODAL, MOUSE, HOMOLOG OF
OCD1	GDB:118846	DISORDER-1; OCD1
OPD2	GDB:131394	SYNDROME
PCK2	GDB:137198	PHOSPHOENOLPYRUVATE CARBOXYKINASE 2, MITOCHONDRIAL; PCK2
PCLD	GDB:433949	POLYCYSTIC LIVER DISEASE; PLD

PCOS1	GDB:1391802	STEIN-LEVENTHAL SYNDROME
PFKM	GDB:120277	GLYCOGEN STORAGE DISEASE VII
PKD3	GDB:127866	KIDNEY DISEASE 3, AUTOSOMAL DOMINANT; PKD3
PRCA1	GDB:342066	PROSTATE CANCER; PRCA1
PRO1	GDB:128585	
PROP1	GDB:9834318	PROPHET OF PIT1, MOUSE, HOMOLOG OF; PROP1
RBS	GDB:118862	ROBERTS SYNDROME; RBS
RFXAP	GDB:9475355	REGULATORY FACTOR X-ASSOCIATED PROTEIN; RFXAP
RP	GDB:9958158	RETINITIS PIGMENTOSA-8
SLC25A6	GDB:125184	ADENINE NUCLEOTIDE TRANSLOCATOR 3; ANT3 ADENINE NUCLEOTIDE TRANSLOCATOR 3, Y-CHROMOSOMAL; ANT3Y
SPG5B	GDB:250333	SPASTIC PARAPLEGIA-5B, AUTOSOMAL RECESSIVE; SPG5B
STO	GDB:439375	CEREBRAL GIGANTISM
SUOX	GDB:5584405	SULFOCYSTEINURIA
TC21	GDB:5573831	ONCOGENE TC21

THM	GDB:439378	FAMILIAL
TST	GDB:134043	RHODANESE; RDS
TTD	GDB:230276	TRICHOTHIODYSTROPHY; TTD

Equivalents:

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

What is claimed is:

1. A method of identifying a compound that modulates premature translation termination or nonsense-mediated mRNA decay, said method comprising:

(a) contacting a member of a library of compounds with a cell containing a first nucleic acid sequence and a second nucleic acid sequence, wherein the first nucleic acid sequence comprises a regulatory element operably linked to a reporter gene and the second nucleic acid sequence comprises a nucleotide sequence with a premature stop codon that encodes a regulatory protein that binds to the regulatory element of the first nucleic acid sequence and regulates the expression of the reporter gene; and

(b) detecting the expression of the reporter gene, wherein a compound that modulates premature translation termination or nonsense-mediated mRNA decay is identified if the expression of the reporter gene in the presence of the compound is altered relative to the expression of the reporter gene in the absence of the compound or the presence of a negative control.

2. A method of identifying a compound that modulates premature translation termination or nonsense-mediated mRNA decay, said method comprising:

(a) contacting a member of a library of compounds with a cell containing a first nucleic acid sequence, a second nucleic acid sequence and a third nucleic acid sequence, wherein (i) the first nucleic acid sequence comprises a nucleotide sequence encoding a first fusion protein comprising a DNA binding domain and a first protein, the nucleotide sequence of the first protein containing a premature stop codon, (ii) the second nucleic acid sequence comprises a nucleotide sequence encoding a second fusion protein comprising an activation domain and a second protein, the second protein interacting with the first protein to produce a regulatory protein, and (iii) the third nucleic acid sequence comprises a regulatory element operably linked to a reporter gene, the expression of the reporter gene being regulated by the binding of the regulatory protein to the regulatory element; and

(b) detecting the expression of the reporter gene, wherein a compound that modulates premature translation termination or nonsense-mediated mRNA decay is identified if the expression of the reporter gene in the presence of

the compound is altered relative to the expression of the reporter gene in the absence of the compound or the presence of a negative control.

3. A method of identifying a compound that modulates premature translation termination or nonsense-mediated mRNA decay, said method comprising:

(a) contacting a member of a library of compounds with a cell containing a first nucleic acid sequence, a second nucleic acid sequence and a third nucleic acid sequence, wherein (i) the first nucleic acid sequence comprises a nucleotide sequence encoding a first fusion protein comprising a DNA binding domain and a first protein, (ii) the second nucleic acid sequence comprises a nucleotide sequence encoding a second fusion protein comprising an activation domain and a second protein, the nucleotide sequence of the second protein containing a premature stop codon and the second protein interacting with the first protein to produce a regulatory protein, and (iii) the third nucleic acid sequence comprises a regulatory element operably linked to a reporter gene, the expression of the reporter gene being regulated by the binding of the regulatory protein to the regulatory element; and

(b) detecting the expression of the reporter gene, wherein a compound that modulates premature translation termination or nonsense-mediated mRNA decay is identified if the expression of the reporter gene in the presence of the compound is altered relative to the expression of the reporter gene in the absence of the compound or the presence of a negative control.

4. A method for identifying a compound that modulates premature translation termination or nonsense-mediated mRNA decay, said method comprising:

(a) contacting a member of a library of compounds with a cell-free translation mixture and a nucleic acid sequence comprising a regulatory element operably linked to a reporter gene, wherein the reporter gene contains a premature stop codon and the cell-free translation mixture is isolated from cells that have been incubated at about 0°C to about 10°C; and

(b) detecting the expression of the reporter gene, wherein a compound that modulates premature translation termination or nonsense-mediated mRNA decay is identified if the expression of the reporter gene in the presence of

the compound is altered relative to the expression of the reporter gene in the absence of the compound or the presence of a negative control.

5. A method for identifying a compound that modulates premature translation termination or nonsense-mediated mRNA decay, said method comprising:

(a) contacting a member of a library of compounds with a cell-free translation mixture and a nucleic acid sequence comprising a regulatory element operably linked to a reporter gene, wherein the reporter gene contains a premature stop codon and the cell-free translation mixture is a S10 to S30 cell-free extract; and

(b) detecting the expression of the reporter gene, wherein a compound that modulates premature translation termination or nonsense-mediated mRNA decay is identified if the expression of the reporter gene in the presence of the compound is altered relative to the expression of the reporter gene in the absence of the compound or the presence of a negative control.

6. The method of claim 4, wherein the cell-free translation mixture is a S10 to S30 cell-free extract.

7. The method of claim 5, wherein the cell-free translation mixture is a S12 cell-free extract.

8. The method of claim 6, wherein the cell-free translation mixture is a S12 cell-free extract.

9. A method of identifying a compound to be tested for its ability to prevent or treat a disease characterized by or associated with the presence of a premature stop codon in a gene, said method comprising:

(a) contacting a member of a library of compounds with a cell containing a nucleic acid sequence comprising a reporter gene with a premature stop codon; and

(b) detecting the expression of the reporter gene,

so that if the expression of the reporter gene in the presence of the compound is altered relative to the expression of the reporter gene in the absence of the compound or the

presence of a negative control, then a compound to be tested for its ability to prevent or treat the disease is identified, wherein the disease is familial hypercholesterolemia, osteogenesis imperfecta, cirrhosis, ataxia telangiectasia or a lysosomal storage disease.

10. A method of identifying a compound to be tested for its ability to prevent or treat a disease characterized by or associated with the presence of a premature stop codon in a gene, said method comprising:

(a) contacting a member of a library of compounds with a cell-free translation mixture and a nucleic acid sequence comprising a reporter gene with a premature stop codon; and

(b) detecting the expression of the reporter gene,

so that if the expression of the reporter gene in the presence of the compound is altered relative to the expression of the reporter gene in the absence of the compound or the presence of a negative control, then a compound to be tested for its ability to prevent or treat the disease is identified, wherein the disease is familial hypercholesterolemia, osteogenesis imperfecta, cirrhosis, ataxia telangiectasia or a lysosomal storage disease.

11. The method of claim 1, 2, 3, 4 or 5, wherein the method further comprises determining the structure of the compound that suppresses premature translation termination or nonsense-mediated mRNA decay.

12. The method of claim 9 or 10, wherein the method further comprises determining the structure of the compound.

13. The method of claim 1, 2, 3, 4, 5, 9 or 10, wherein the reporter gene is firefly luciferase, renilla luciferase, click beetle luciferase, green fluorescent protein, yellow fluorescent protein, red fluorescent protein, cyan fluorescent protein, blue fluorescent protein, beta galactosidase, beta glucoronidase, beta lactamase, chloramphenicol acetyltransferase, or alkaline phosphatase.

14. The method of claim 1, 2, 3 or 9, wherein the cell is selected from the group consisting of 293T, HeLa, MCF7, Wi-38, SkBr3, Jurkat, CEM, THP1, 3T3, and Raw264.7 cells.

15. The method of claim 4, 5 or 10, wherein the cell-free translation mixture is a cell-free extract from 293T, HeLa, MCF7, Wi-38, SkBr3, Jurkat, CEM, THP1, 3T3, or Raw264.7 cells.

16. The method of claim 1, 2, 3, 4, 5, 9 or 10, wherein the compound is selected from a combinatorial library of compounds comprising peptoids; random biooligomers; diversomers such as hydantoins, benzodiazepines and dipeptides; vinylogous polypeptides; nonpeptidal peptidomimetics; oligocarbamates; peptidyl phosphonates; peptide nucleic acid libraries; antibody libraries; carbohydrate libraries; and small organic molecule libraries.

17. The method of claim 16, wherein the small organic molecule libraries are libraries of benzodiazepines, isoprenoids, thiazolidinones, metathiazanones, pyrrolidines, morpholino compounds, or diazepindiones.

18. The method of claim 1, 2, 3, 4, 5, 9 or 10, wherein the premature stop codon is UAG or UGA.

19. The method of claim 1, 2, 3, 4, 5, 9 or 10, wherein the premature stop codon context is UAGA, UAGC, UAGG, UAGU, UGAA, UGAC, UGAG or UGAU.

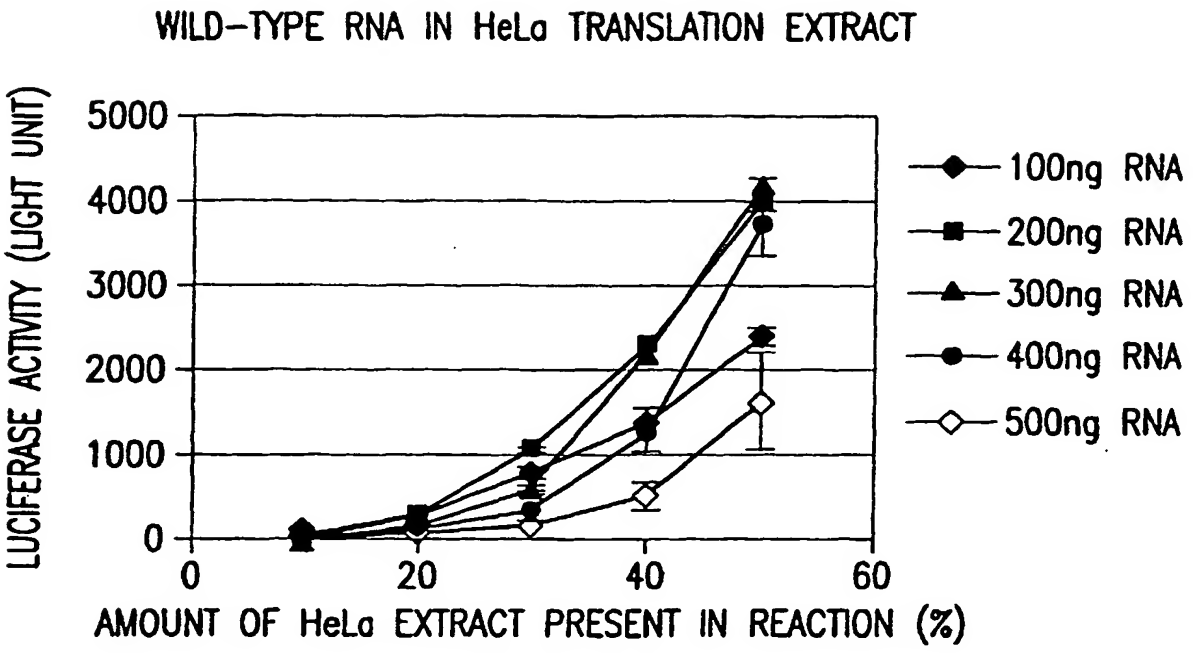

WILD-TYPE RNA IN HeLa TRANSLATION EXTRACT
LUCIFERASE ACTIVITY (LIGHT UNIT)
5000
4000
3000
2000
1000
0
0
20
40
60
AMOUNT OF HeLa EXTRACT PRESENT IN REACTION (%)
100ng RNA
200ng RNA
300ng RNA
400ng RNA
500ng RNA

FIG. 1

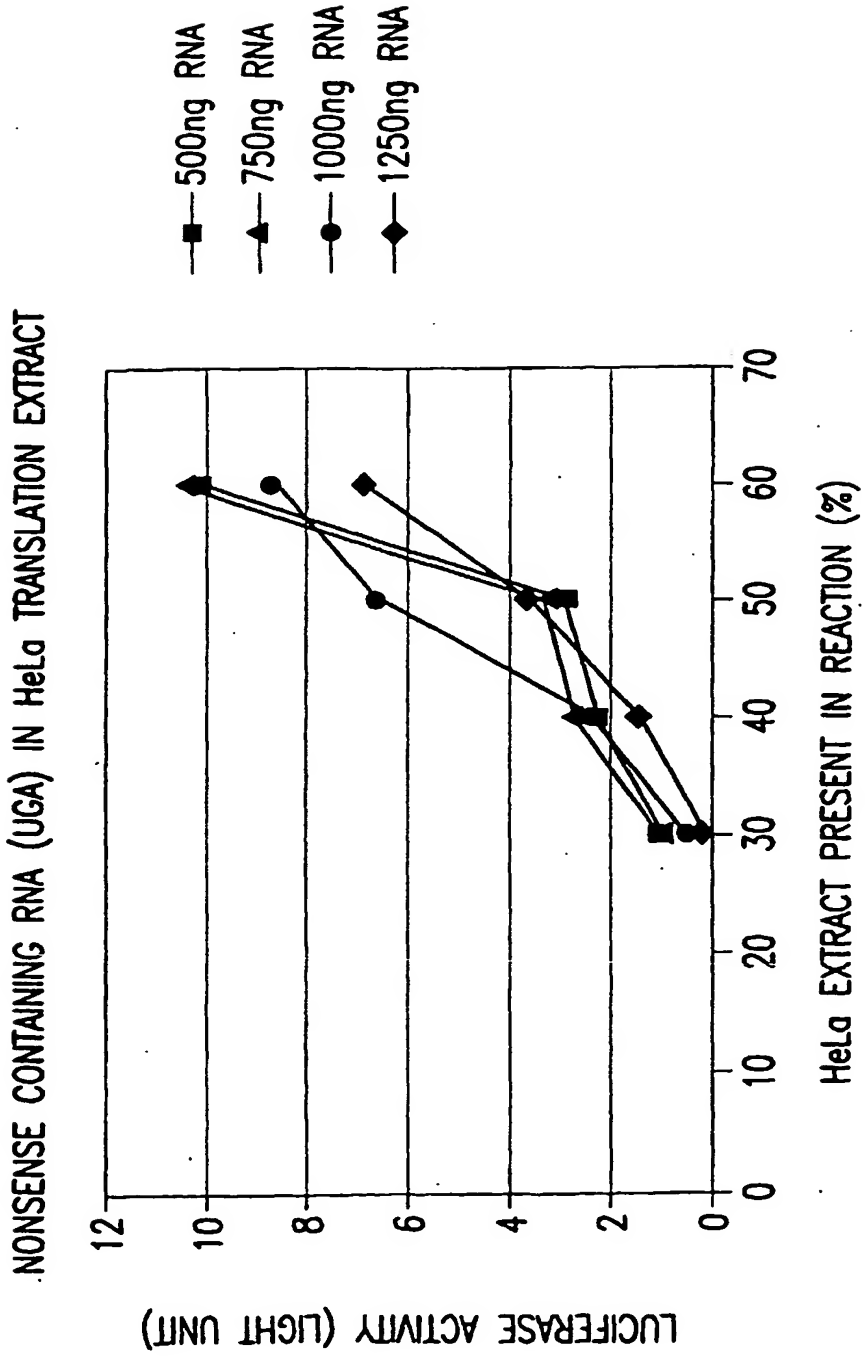

500ng RNA
750ng RNA
1000ng RNA
1250ng RNA
NONSENSE CONTAINING RNA (UGA) IN HeLa TRANSLATION EXTRACT
LUCIFERASE ACTIVITY (LIGHT UNIT)
0
2
4
6
8
10
12
0
10
20
30
40
50
60
70
HeLa EXTRACT PRESENT IN REACTION (%)

FIG.2

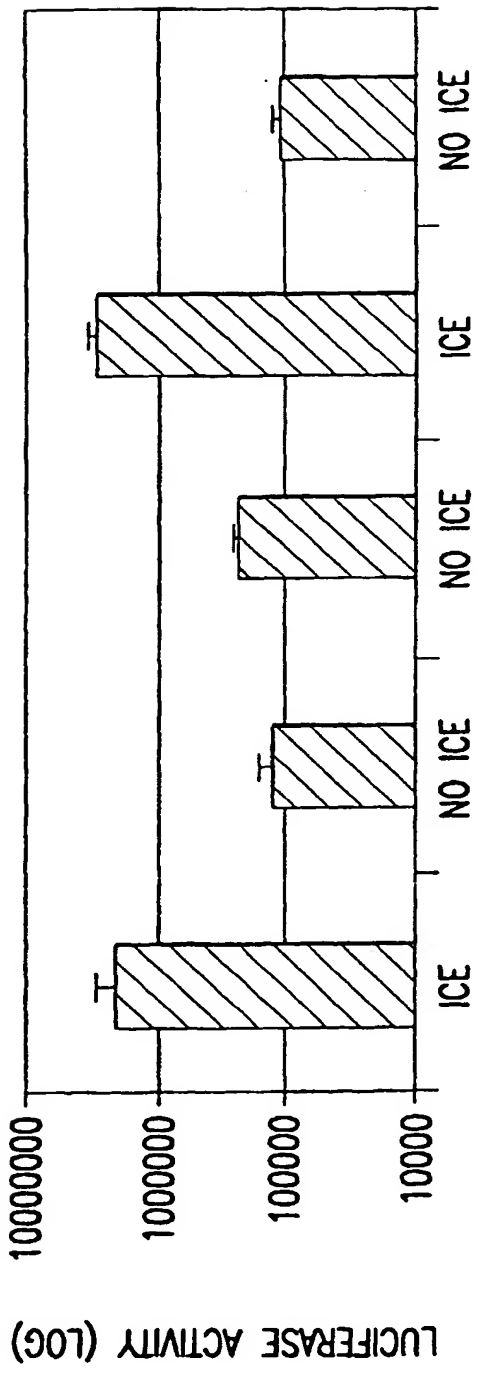

LUCIFERASE ACTIVITY (LOG)
10000000
1000000
100000
10000
ICE
NO ICE
NO ICE
ICE
NO ICE

FIG.3

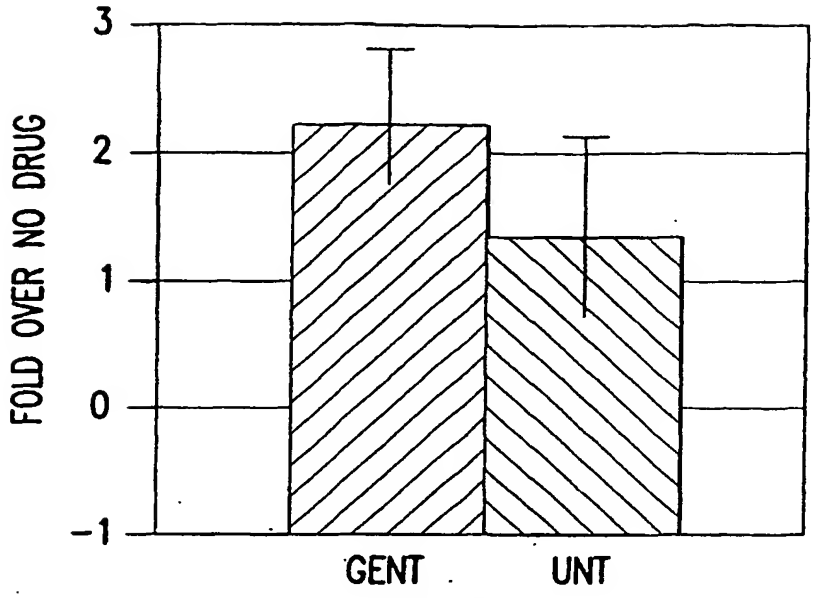


FIG.4

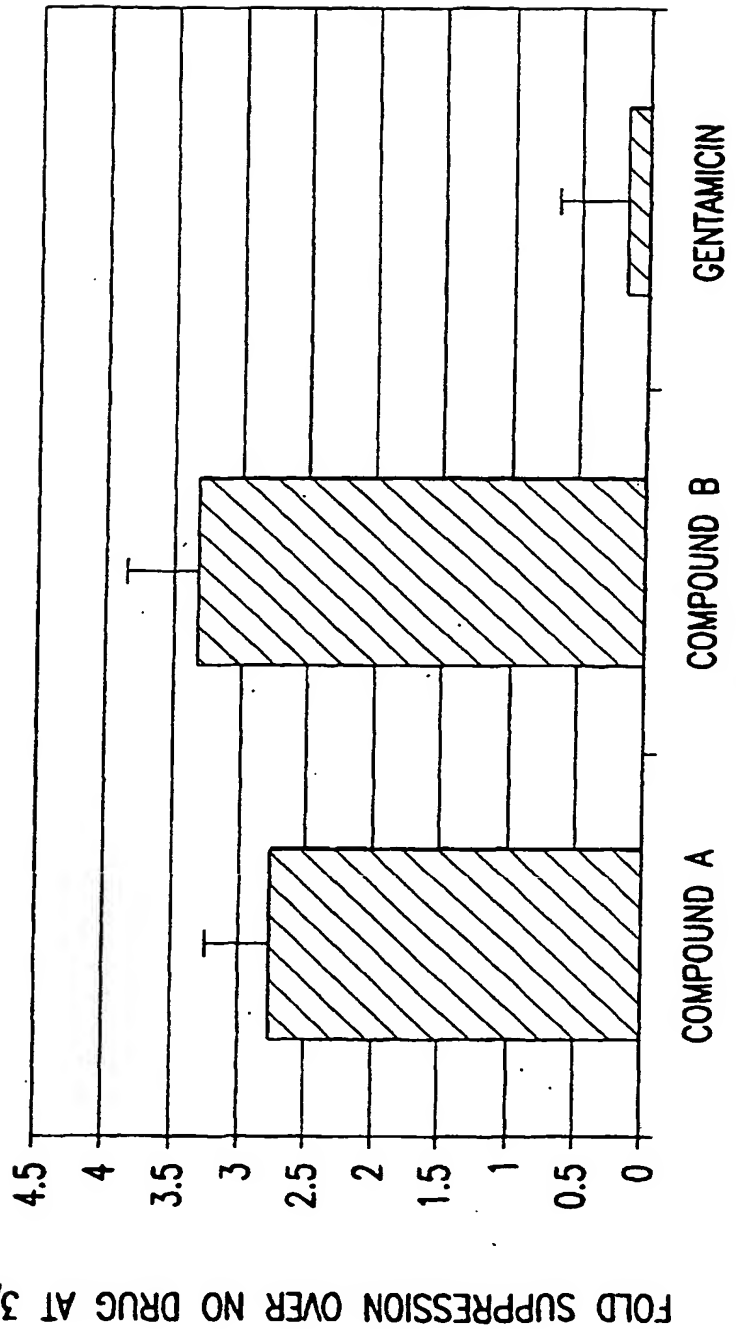

FOLD SUPPRESSION OVER NO DRUG AT 3μM
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
COMPOUND A
COMPOUND B
GENTAMICIN

FIG.5

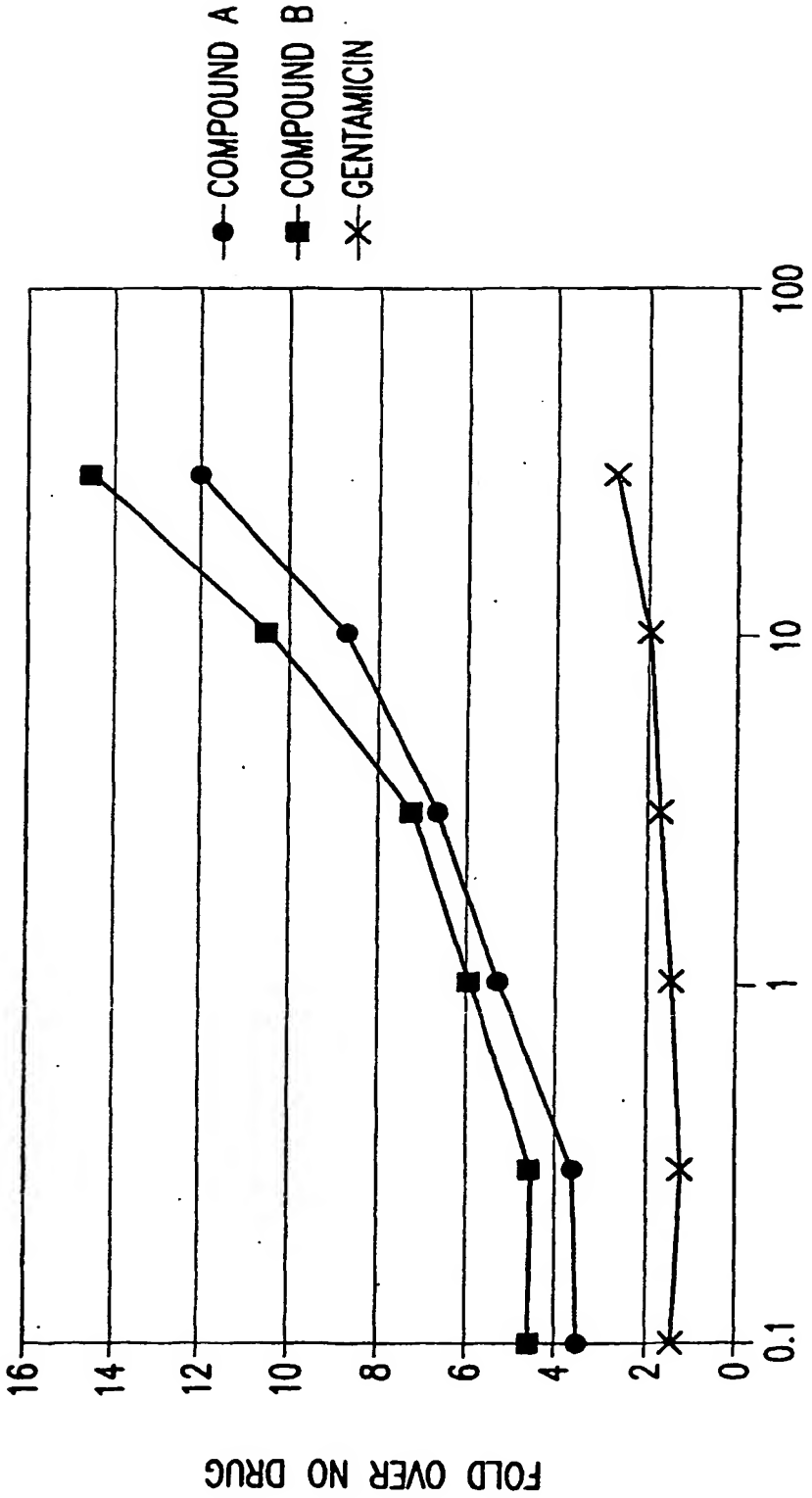


FIG.6A

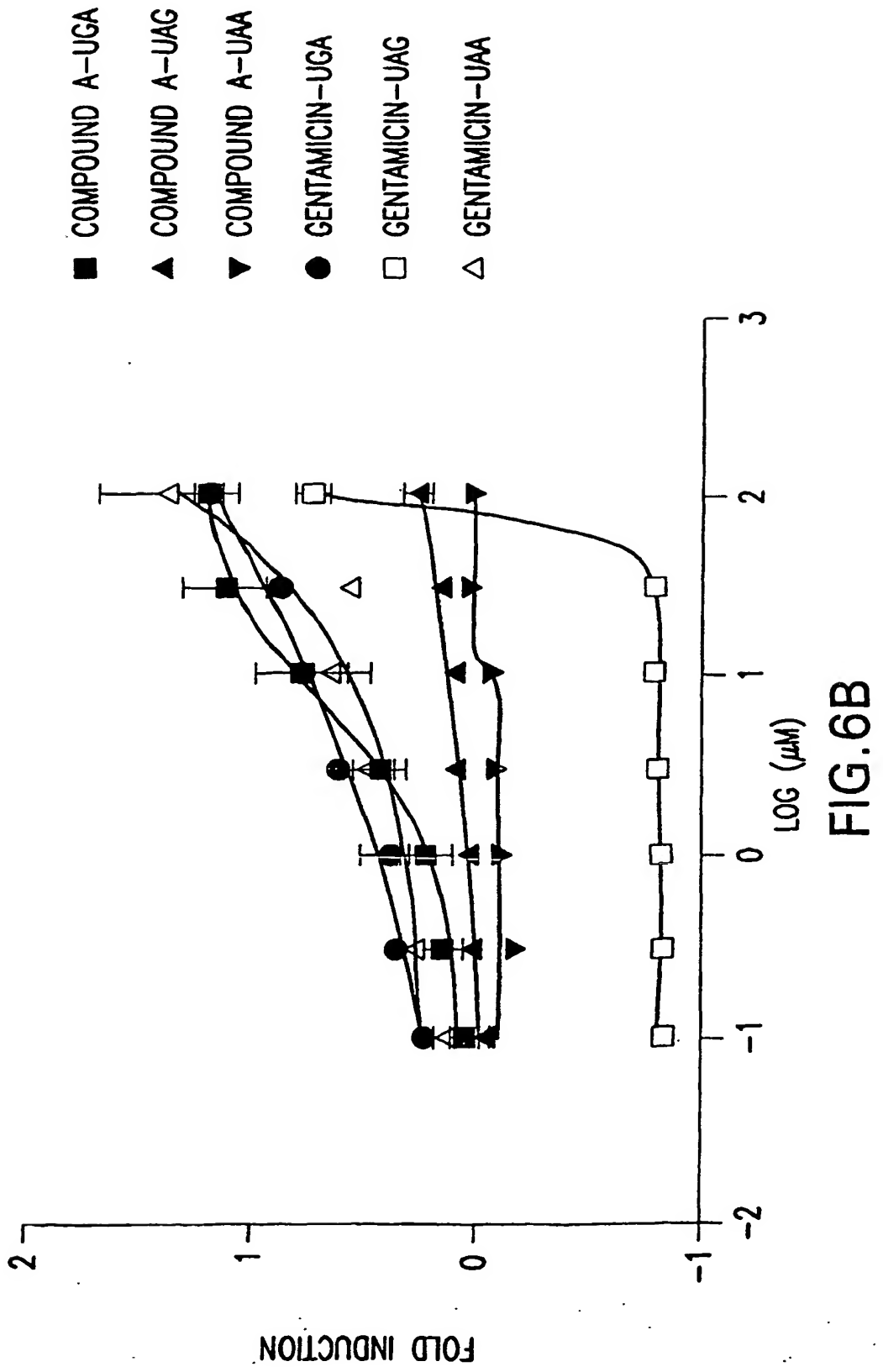

COMPOUND A-UGA
COMPOUND A-UAG
COMPOUND A-UAA
GENTAMICIN-UGA
GENTAMICIN-UAG
GENTAMICIN-UAA
FOLD INDUCTION
2
1
0
-1
-2
3
2
1
0
-1
LOG (μM)

FIG.6B

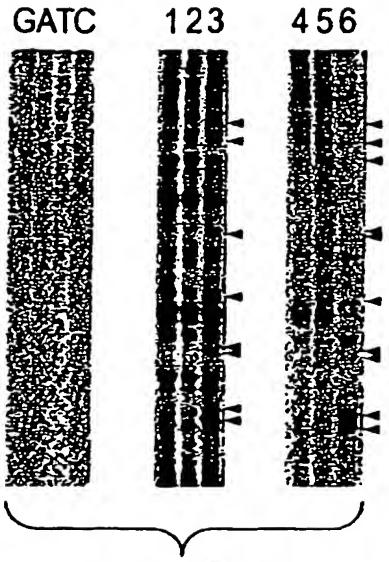


FIG.7A

FIG.7B

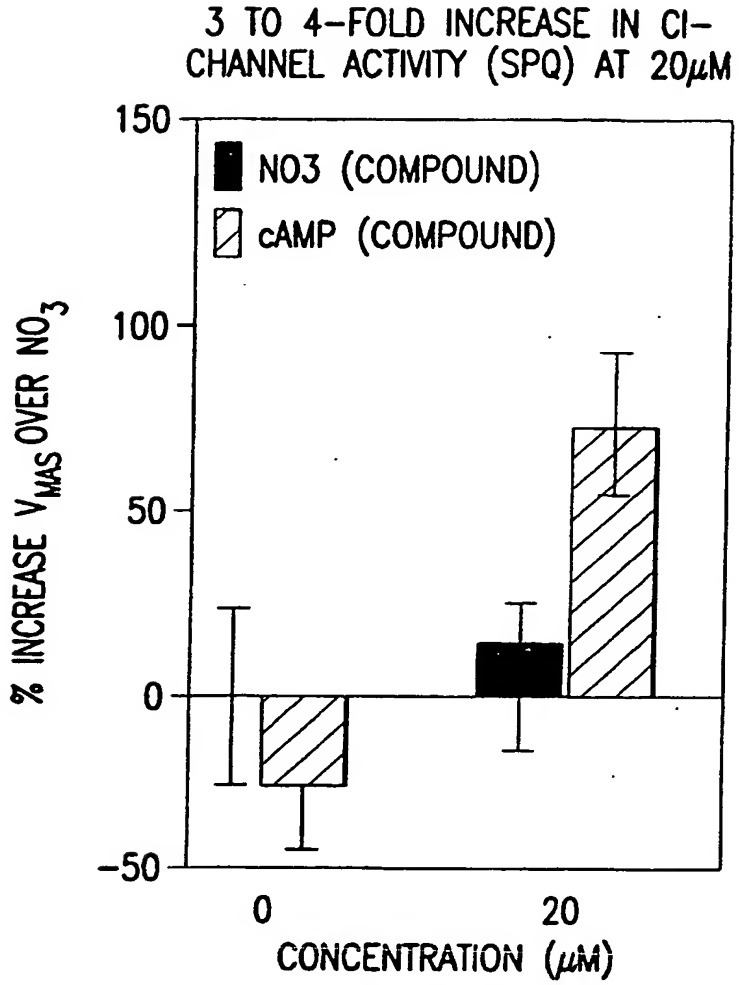

3 TO 4-FOLD INCREASE IN Cl- CHANNEL ACTIVITY (SPQ) AT 20μM
150
100
50
0
-50
NO3 (COMPOUND)
cAMP (COMPOUND)
% INCREASE V_{MAS} OVER NO_3
0
20
CONCENTRATION (μM)

FIG.8

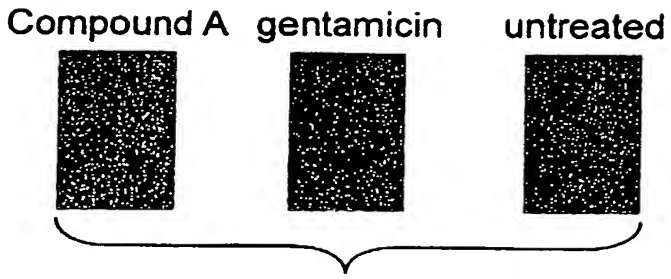


FIG.9

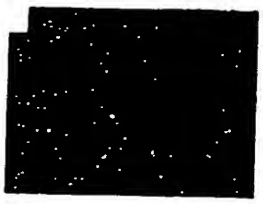


FIG.10A

FIG.10B

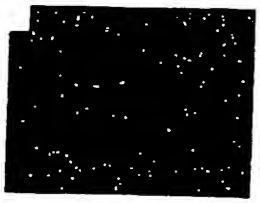


FIG.10C

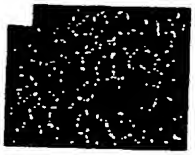


FIG.10D

FIG.10E

FIG.10F